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Review

Correlation of retention and selectivity of separation in reversed-phase high-performance liquid chromatography with interaction indices and with lipophilic and polar structural indices

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ABSTRACT

A simple semi-empirical model of interaction indices proposed to describe the retention in reversed-phase systems, assuming a predominant role of the interactions in the mobile phase to control the retention, and the concept of lipophilic and polar indices derived on its basis are reviewed. Specific contributions of the stationary phase to the retention are neglected to a first approximation. The model suggests that the polarities and the concentrations of the mobile phase components and the volume and polarity of the solute, characterized by its molar volume and interaction index, respectively, are the major factors controlling the retention. Based on this model, the retention and selectivity in reversed-phase systems can be understood to be comprised of lipophilic and polar contributions, which are characterized quantitatively by constants related to the structure of the solutes, *i.e.*, by the lipophilic and polar indices n_{ce} and q_i, respectively. These indices can be used to characterize and predict the retention and selectivity in reversed-phase chromatography using a retention scale calibrated with a suitable reference homologous series, the first member of which can serve as the selectivity reference standard. The two-indices approach allows the use of a single calibration equation over a wide range of compositions of a binary mobile phase with better precision of prediction of retention than single-index calibration approaches. It is also possible to characterize and predict the retention and separation selectivity in ternary mobile phases composed of two different organic solvents in water and in gradient elution chromatography. It has been verified for several types of compounds that the indices can be calculated from the additive increments of structural substituents. The present method allows the prediction of possible changes in the order of elution with changing composition of the mobile phase and optimization of the separation conditions. It was used for a quantitative description of retention in homologous and oligomeric series, where it was possible to explain and predict conditions under which some polar oligomeric series are eluted in order of decreasing molecular mass.

CONTENTS

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1. INTRODUCTION

Various models have been proposed to describe the retention in reversed-phase systems, for example a competitive adsorption model with a modified scale of the solvent strength [1,2], a model based on the probability of interactions of the solute in the stationary and mobile phases [3,4], modified models of the distribution of a solute between two liquid phases such as the model using the Hildebrand solubility parameter theory [5–8], or the model supported by the concept of molecular connectivity [9], and rigorous models based on the solvophobic theory [10,11] or on the molecular statistical theory [12].

Unfortunately, sophisticated models introduce a number of physico-chemical constants which are often not known or are difficult and time consuming to determine, so that such models are not very suitable for rapid prediction of retention data.

The most characteristic feature of reversed-phase chromatography is a lower polarity of the stationary phase in comparison with the mobile phase. Theoretically, alkyl phases chemically bonded on a suitable support such as octadecylsilica or octylsilica materials commonly used in contemporary practice of reversed-phase HPLC should behave as almost ideally non-polar materials with the properties of long-chain aliphatic hydrocarbons.

In reality, the bonded alkyl chains differ from the free molecules of hydrocarbons in the liquid state by their limited mobility. Further, for steric reasons it is virtually impossible to modify all the available silanol groups on the surface of silica gel by chemical reaction with the silanization reagent and the unreacted silanol groups may affect the retention by specific interactions, especially with basic solutes. Finally, organic solvents used as the components of the mobile phases in reversed-phase systems are preferentially sorbed by the stationary phase and can significantly modify its properties [13,14].

The solvophobic theory emphasizes the importance of the mobile phase interactions in the control of the retention mechanism [10,11]. The solvophobic interactions are understood as the driving force of the formation of associates of the solutes with the non-polar stationary phase. The retention results from a decrease in the contact area of the solute with the mobile phase connected with its transition from the bulk mobile phase to the surface of the stationary phase. Replacement of weaker interactions between the moderately polar solute and polar mobile phase by mutual interactions between the strongly polar molecules of the mobile phase in the space element of the mobile phase occupied by a solute molecule before the transition results in an overall energy decrease in the system, which is the driving force of the retention in the absence of strong (polar) interactions of the solute with the stationary phase.

We found the theoretical background of this model of retention useful as the starting point in the derivation of a simplified semi-empirical description of reversed-phase systems making it possible to characterize and predict the retention and selectivity within a series of compounds to be separated [15]. Because of simplifications concerning the effects of the stationary phase accepted in the derivation, this approach can be applied for relative rather than absolute predictions of retention and selectivity and a suitable set of standard reference compounds is necessary to calibrate the retention (or selectivity) scale.

The predictive model derived in this way is based on interaction indices and is described in

Section 2. This model was used for general characterization of the retention in homologous and oligomeric series, as described in Section 3. This section should facilitate understanding of the phenomenological approach based on lipophilic and polar indices for the characterization of retention and selectivity over a wide range of mobile phase compositions, using a suitable homologous series as the set of reference calibration standards, which is described in Section 4.

2. CHARACTERIZATION OF RETENTION USING INTERACTION INDICES

2.1. Derivation of the retention equation

Theoretically, if the stationary phase is ideally non-polar the dispersive forces account for the non-polar interactions between the stationary phase and the solutes. These interactions are non-specific and occur also in the mobile phase where, in addition, polar interactions (dipole–dipole, proton–donor and proton–acceptor) become effective between the molecules of the solute and those of the mobile phase on the one hand and mutual interactions between the polar molecules of the mobile phase on the other. A more or less strong repulsion results of the solute molecules from the mobile phase into the stationary phase, which behaves as a passive acceptor rather than a source of active attraction forces for the sorbed molecules, in contrast to the sorption on polar adsorbents. This is, of course, only a first approximation, a rough and imperfect scheme of complex phenomena controlling the retention, in which the role of the stationary phase cannot be neglected. Therefore, a description of retention based only on the interactions in the mobile phase is not rigorous and its value for an absolute prediction of retention surely would be questionable.

Assuming that the effect of the non-polar interactions between the solute and the stationary phase is approximately equilibrated by the effect of the non-polar interactions between the solute and the mobile phase and that no polar adsorption sites are present in the stationary phase, the retention is controlled mainly by the polar interactions in the mobile phase. The main driving force of retention can be understood as the difference in the free energy of polar interactions between the molecules of the mobile phase, ΔG_{M-M}, and the free energy of polar, usually weaker, interactions between the molecules of the solute and the molecules of the mobile phase, ΔG_{M-X}. As the sample solutes are separated in dilute solutions in analytical HPLC, there is no need to consider mutual interactions between solute molecules. The energy of polar interactions between two molecules is characterized as the product of the contributions from each molecule. The individual contribution is directly proportional to the index of interaction, I_i, characterizing the polarity of the molecule i. As every solute is characterized by unique value of I_i, the proportionality constant, $c_i > 0$, accounts for the specific character of the polar interactions connected with the type of the organic solvent used in the mobile phase and affects the constants of the calibration equation of the interaction indices scale based on a set of reference standards [15]. As one molecule of the solute A can interact with different number of molecules of the compound B, it is necessary to relate the interaction indices to the unit volume element of the space where the interactions occur. According to this definition, the interactions connected with the transfer of one molecule of the solute X from the mobile to the stationary phase occur in a volume proportional to V_X, the molar volume of the solute. Then $\Delta G_{M-X} = c_M I_M c_X I_X V_X$ and $\Delta G_{M-M} = c_M I_M c_M I_M V_X$, where I_X is the interaction index of the solute and I_M that of the mobile phase and $c_X > 0$, $c_M > 0$ are c_i of the solute and of the mobile phase, respectively. Neglecting the relatively small entropic contribution to the retention, we obtain the following equation for the total change of the free energy of transfer, $-\Delta G$, of one mole of the solute from the mobile to the stationary phase [15]:

$$\begin{aligned} -\Delta G &= -(\Delta G_{M-M} - \Delta G_{M-X}) \\ &= 2.31RT\,(\log k' - \log \phi) \\ &= c_M V_X I_M (c_M I_M - c_X I_X) \end{aligned} \quad (1)$$

where k' is the capacity factor of the solute, R is

the gas constant, T is absolute temperature and $\phi = V_S/V_M$ is the phase ratio in the column, *i.e.*, the ratio of the volumes of the stationary phase, V_S, and of the mobile phase, V_M. V_S is defined in this work as the total volume of the packing material in the column, $V_S = V_G - V_M$, where V_G is the geometrical inner volume of the empty column.

Eqn. 1 is formally similar to the retention equation derived on the basis of Hildebrand solubility parameters [5–7], but the solubility parameters δ are absolute quantities and the interaction indices are defined relative to a calibration series of reference standards. Another difference from the solubility parameter approach is in neglecting the interactions in the stationary phase, which are accounted for by the solubility parameter of the stationary phase, δ_S. However, δ_S is poorly defined and as it is impossible to determine experimentally, it is usually approximated by δ of *n*-alkanes [6,7].

In binary mobile phases, I_M can be expressed as a linear function of the concentration of the organic solvent, φ, in analogy to Snyder's polarity indices P' [16]:

$$I_M = (1 - \varphi)I_{H_2O} + \varphi I_{org} \quad (2)$$

where I_{H_2O} and I_{org} are the interaction indices of water and of the organic solvent, respectively; φ is given as the volume fraction of the organic solvent [%(v/v) · 10^{-2}] in this work, so that $0 \leq \varphi \leq 1$. The mean values of the interaction indices of the most common solvents used in reversed-phase chromatography determined in various systems [15] are given in Table 1.

TABLE 1

VALUES OF THE INTERACTION INDICES I_{H_2O} AND I_{org} FOR COMMONLY USED SOLVENTS IN REVERSED-PHASE CHROMATOGRAPHY (FROM REF. 15)

MeOH = Methanol; ACN = acetonitrile; DIOX = dioxane; THF = tetrahydrofuran.

I_{H_2O}	$I_{org(MeOH)}$	$I_{org(ACN)}$	$I_{org(DIOX)}$	$I_{org(THF)}$
47	21.1	18.3	18.8	11.4

Combining eqns. 1 and 2, we obtain the retention equation for the dependence of the capacity factor of the solute, k', on the composition of the mobile phase [15]:

$$\log k' = a - m\varphi + d\varphi^2 \quad (3)$$

This quadratic equation is formally identical with the retention equation derived earlier both on the basis of the solubility parameter theory for liquid–liquid and reversed-phase systems [5–7] and using the molecular statistical theory [12], but the constants a, m, d have different meanings here:

$$a = \log \phi + \frac{V_X I_{H_2O} c_M}{2.31RT}(I_{H_2O}c_m - I_X c_X) \quad (4)$$

$$m = \frac{V_X c_M}{2.31RT}(2I_{H_2O}c_M - I_X c_X)(I_{H_2O} - I_{org}) \quad (5)$$

$$d = \frac{V_X c_M^2}{2.31RT}(I_{H_2O} - I_{org})^2 \quad (6)$$

The value of the quadratic term $d\varphi^2$ in eqn. 3 often is not very significant and can be neglected to first approximation, at least for lower values of φ. The retention equation is then reduced to the well known and widely used form [5,8,17]:

$$\log k' = a - m\varphi \quad (7)$$

Eqn. 6 predicts that the relative importance of the quadratic term should increase with decreasing polarity of the organic solvent, *i.e.*, with increasing difference $I_{H_2O} - I_{org}$. This is in agreement with the experimental observations, where the parameters d are significantly lower in methanol–water than in acetonitrile–water mobile phases (see, *e.g.*, Table 2 [18]). The log k' *versus* φ plots are often linear in aqueous solutions of methanol ($I_{org} = 21.1$), slightly nonlinear in acetonitrile–water mixtures ($I_{org} = 18.3$) and significantly curved in mobile phases containing tetrahydrofuran in water ($I_{org} = 11.4$).

It should be noted that the intercept and the slope of a regression line fitted to the experimental data points correspond to the constants a and m as defined by eqns. 4 and 5 only if the term $d\varphi^2$ is sufficiently low (<5 to 10% of $a + m\varphi$).

If the contribution of the quadratic term to retention is not significant, eqn. 5 predicts that

TABLE 2

VALUES OF THE PARAMETERS m AND d OF EQN. 3 IN METHANOL–WATER (60–90%) AND ACETONITRILE–WATER (50–80%) MOBILE PHASES

Column: Silasorb C_8, 7 μm (300 × 3.8 mm I.D.) (from Ref. 18).

Solute	CH_3OH–water		CH_3CN–water	
	m	d	m	d
Benzonitrile	2.64	0.0	3.07	0.6
Anisole	2.85	0.0	3.78	1.1
Acetanilide	5.56	1.9	7.46	4.5
n-Propyl phenyl ether	4.02	0.3	5.50	2.2
Ethyl benzoate	3.80	0.2	5.23	2.0
Benzaldehyde	4.89	1.3	6.05	3.0
Styrene	3.75	0.2	5.10	1.9
α,α,α-Trichlorotoluene	6.79	1.7	9.18	4.7
Dibutyl ether	5.83	1.3	8.00	4.1
1-Bromobutane	4.06	0.4	5.56	2.3

the parameter m should be directly proportional to the difference between the interaction indices of water and of the organic solvent, $(I_{H_2O} - I_{org})$. Consequently, m is expected to increase with decreasing I_{org}, *i.e.*, with decreasing polarity of the organic solvent, which agrees with the experimental data in Table 2 evaluated according to eqn. 3.

As in this simplified model of retention the role of the stationary phase has been neglected, it appears only in the value of the phase ratio $\phi = V_S/V_M$ in eqn. 4. The volume of the stationary phase V_S increases and consequently V_M decreases with the amount and the length of the bonded alkyl chains. The retention of a solute has been found experimentally to increase with increasing length of the bonded alkyl only up to a certain "critical chain length" [19] and because of the simplifying assumptions accepted in the derivation of the retention eqn. 1, eqns. 3–7 do not allow any meaningful conclusions concerning the effects of the stationary phase on retention.

The effect of the structure of the solute on retention is expected at two levels. The retention should decrease with increasing intensity of polar interactions, *i.e.*, with the values of the interaction indices, I_X. On the other hand, it should increase with the volume in which the interactions occur, which is expected to be approximately proportional to the molar volume of the solute, V_X. With increasing size of the non-polar part in the molecule of a sample compound, both the slope and the curvature of the log k' *versus* φ plots are expected to increase (eqns. 5 and 6) and the experimental observations are in agreement with this prediction. Fig. 6 shows examples of increasing slopes of the log k' *versus* φ plots with increasing length of alkyl chains in the molecules of the solutes. The curvature of these plots increases with increasing parameter d in eqn. 3 and this parameter has been found experimentally to increase, *e.g.*, from 1.88 for ethylbenzene to 3.94 for *n*-hexylbenzene and from 3.67 for *n*-heptane to 4.72 for *n*-decane [15]. Only the effects of the size of the structural units of low and approximately equal polarities can be compared in this way, otherwise the behaviour is complicated by the effects of changing polarities.

The approach similar to the description of retention in binary mobile phases can be applied also to multi-component solvent systems, where eqn. 2 becomes:

$$I_M = \left(1 - \sum \varphi_i\right) I_{H_2O} + \sum \varphi_i I_{org,i}$$

$$= I_{H_2O} - \sum \varphi_i (I_{H_2O} - I_{org,i}) \quad (8)$$

where $I_{org,i}$ and φ_i are the interaction index and the concentration of the organic solvent i in the aqueous–organic multi-solvent mobile phase, respectively. From eqns. 1 and 8, the following equation was derived for k' [20]:

$$\log k' = a - \sum m_i \varphi_i + \sum d_i \varphi_i^2 + 2 \sum \sqrt{d_i d_j} \varphi_i \varphi_j \quad (9)$$

For a ternary mobile phase containing organic solvents X and Y in concentrations φ_X, φ_Y in water, eqn. 9 becomes [20]:

$$\log k' = a - m_X \varphi_X - m_Y \varphi_Y + d_X \varphi_X^2 + d_Y \varphi_Y^2 + 2\sqrt{d_X d_Y} \varphi_X \varphi_Y \quad (10)$$

where the constants a, m_X, m_Y, d_X and d_Y are

defined by eqns. 4–6, with either $I_{org} = I_{org,X}$, or $I_{org} = I_{org,Y}$.

The contributions of the terms with d_X, d_Y to log k' often can be neglected over a limited range of the concentrations φ_x, φ_y and the retention can be calculated from the following simple equation [20–22]:

$$\log k' = a - m_X\varphi_X - m_Y\varphi_Y \quad (11)$$

The values of the constants a, m_X, m_Y and, if necessary, d_X and d_Y can be determined in binary mobile phases, which makes it possible to predict the retention in ternary mobile phases X–Y–water from the retention data in binary mobile phases X–water and Y–water, using eqn. 10 or eqn. 11. When applying this approach, we often find significant differences in the values of the constant a determined by regression of the log k' *versus* φ plots for the individual binary mobile phases containing different organic solvents X and Y, $a_X \neq a_Y$, which would make the use of eqn. 10 or 11 difficult. A simple but efficient empirical remedy is to use the weighted average of the two experimentally found values for a [22]:

$$a = \frac{a_X\varphi_X + a_Y\varphi_Y}{\varphi_X + \varphi_Y} \quad (12)$$

The errors of the k' predicted from the data in binary mobile phases using eqns. 10 or 11 and 12 are usually 5% or less [21,22]. Eqns. 11 and 12 were used as the basis for calculations of retention volumes in reversed-phase chromatography using elution with ternary gradients. For more details, see Ref. 21.

2.2. Calibration of the retention scale

The retention equation derived on the basis of the interaction indices approach predicts the effects of the individual parameters (I_X, I_{org}, V_X) on the retention in reasonable qualitative agreement with the experimentally observed behaviour. To permit the use of this equation for quantitative calculations, the values of the interaction indices I_X should be known. For this purpose, a calibration scale based on several adequately chosen standard compounds must be defined. To make the calibration easier, eqns. 3–7 are rearranged into the form

$$\log k^* = \frac{\log k' - \log \phi}{V_X} = A - BI_X \quad (13)$$

where

$$A = A'_0 - A'_1\varphi \quad (14)$$

$$B = B'_0 - B'_1\varphi \quad (15)$$

(neglecting the second-order terms with respect to φ) and

$$A'_0 = \frac{c_M^2 I_{H_2O}^2}{2.31RT} \quad (16)$$

$$A'_1 = \frac{2c_M^2 I_{H_2O}(I_{H_2O} - I_{org})}{2.31RT} \quad (17)$$

$$B'_0 = \frac{c_M c_X I_{H_2O}}{2.31RT} \quad (18)$$

$$B'_1 = \frac{c_M c_X (I_{H_2O} - I_{org})}{2.31RT} \quad (19)$$

Eqn. 13 predicts a linear variation of the logarithms of the specific capacity factors, k^*, with the interaction indices of the solutes in a mobile phase of a constant composition. If the plots of log k' *versus* φ are linear over the range of mobile phase compositions of interest, eqns. 14 and 15 can be used to calculate the constants A and B.

The interaction indices of the initially selected calibration standards, namely benzene, toluene, nitrobenzene, acetophenone and anisole, were obtained for each calibration standard by slight modification of its polarity index, P' [16], as the arithmetic mean of the values calculated by linear regression of the log k^* *versus* P' dependencies in fifteen mobile phases with different concentrations of methanol, acetonitrile, tetrahydrofuran and dioxane in water [15].

Later, various groups of calibration standards were tested for least deviations from the log k^* *versus* I_X regression curves in different mobile phases and based on these experiments, a new series of calibration standards was selected: 1,4-dichlorobenzene ($I_X = 1.05$), 3-chlorotoluene ($I_X = 2.20$), 3-bromonitrobenzene ($I_X = 3.44$), nitrobenzene ($I_X = 4.49$) and benzonitrile ($I_X =$

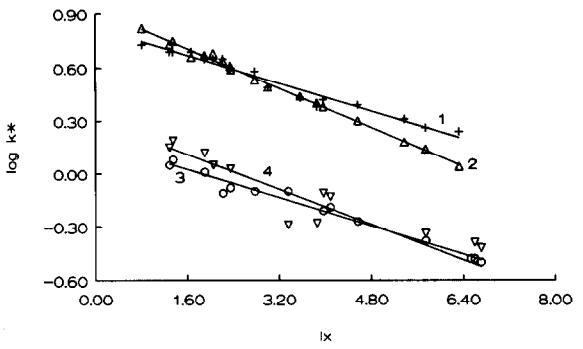

Fig. 1. Plots of the logarithms of specific capacity factors, k^*, of various aromatic compounds in mobile phases composed of acetonitrile–water, (1) 50:50 and (3) 80:20, and of methanol-water, (2) 60:40 and (4) 80:20, as a function of the interaction indices, I_X. Column: Silasorb C_8, 7.5 μm (300 × 3.8 mm I.D.). The interaction indices were determined as the arithmetic means of the values determined on a Silasorb C_{18} column in seventeen mobile phases containing various concentrations of methanol, acetonitrile or tetrahydrofuran in water, using 1,4-dichlorobenzene, 3-chlorotoluene, 3-bromonitrobenzene, nitrobenzene and benzonitrile as the calibration standards for the I_X scale.

5.32) [23]. The relative standard deviations for I_X of these standards were between 4.2 and 6.8%. Fig. 1 illustrates the validity of eqn. 13 based on these calibration standards for a variety of aromatic compounds with different functional groups on an octylsilica column in four mobile phases. The fit of the calibration graphs to the experimental data is better for mobile phases with lower concentrations of the organic solvents such as 50% acetonitrile or 60% methanol in water (lines 1 and 2 in Fig. 1) than in mobile phases containing 80% or more of methanol or acetonitrile (lines 3 and 4 in Fig. 1), where the more significant scatter of the experimental data can be attributed to the lower precision of the determination of k', because relatively small errors in V_R or V_M result in large errors in k^*.

With the constants A and B determined by linear regression of the dependences of the experimental log k^* on the I_X of the calibration standards in a given mobile phase, eqn. 13 can be used to determine the I_X of sample solutes from their capacity factors k'. If we know the constants A and B for another mobile phase, the interaction indices of the sample solutes can be used for prediction of their k' in this mobile phase.

The interaction indices approach can be used not only in pure aqueous–organic mobile phases, but also in mobile phases containing neutral electrolytes as additives. This is demonstrated by the agreement of the values of the interaction indices in 60% methanol and in the mobile phases with the addition of lithium sulphate (Table 3) [18]. The interaction indices can be significantly affected in the presence of the electrolytes taking part in secondary equilibria in the chromatographic system, such as in the formation of ion pairs or in pH controlled acid–base equilibria.

The capacity factors predicted using the interaction indices usually agree with the experimental values to within 10–15% relative, but occasionally the deviations may exceed 20%, mainly for basic compounds, probably because of the effect of the stationary phase interactions, neglected in the present simple model [24].

An attempt was reported to account for the effects of the entropic changes during the transition of the solutes between the mobile and stationary phases by introduction of additional terms into eqn. 3 [25], but this equation then becomes too complex for easy practical applications.

2.3. Characterization of the separation selectivity

Eqns. 13–15 can be used to characterize the separation selectivity, *i.e.*, the relative retention of two solutes i and j, $r_{i,j} = k'_j/k'_i$ [23]:

$$\log r_{i,j} = A'_0(V_{Xj} - V_{Xi}) - B'_0(V_{Xj}I_{Xj} - V_{Xi}I_{Xi}) - [A'_1(V_{Xj} - V_{Xi}) - B'_1(V_{Xj}I_{Xj} - V_{Xi}I_{Xi})]\varphi \quad (20)$$

The subscripts i,j, in eqn. 20 denote the parameters V_X, I_X of compounds i and j, respectively.

This equation suggests that the separation selectivity of a pair of compounds in reversed-phase systems depends on the differences in both the size and polarity of the two compounds. For compounds of similar polarities, $I_{Xj} \approx I_{Xi}$, the

TABLE 3

INTERACTION INDICES I_X DETERMINED EXPERIMENTALLY IN MOBILE PHASES WITH AND WITHOUT ADDITION OF A SALT

Column as in Table 1 (from Ref. 18).

Solute	Mobile phase[a]		
	60% CH_3OH $A = 1.165$ $B = 0.160$	0.04 M Li_2SO_4 in 60% CH_3OH $A = 1.088$ $B = 0.153$	0.2 M Li_2SO_4 in 60% CH_3OH $A = 1.123$ $B = 0.152$
Benzonitrile	5.38	5.37	5.38
Anisole	4.08	4.18	4.16
Acetophenone	5.35	5.34	5.35
Nitrobenzene	4.50	4.40	4.41
1,4-Dichlorobenzene	0.87	0.76	0.81
3-Bromonitrobenzene	3.21	3.12	3.20
3-Chlorotoluene	1.83	1.80	1.85
Benzene	3.30	3.42	3.34

[a] A and B are the constants in eqn. 13.

separation occurs mainly on the basis of the differences in the size between the molecules of i and j ($V_{Xj} > V_{Xi}$) and the separation selectivity usually decreases with increasing concentration of the organic solvent, φ, in the mobile phase, the selectivity change per unit concentration being directly proportional to the difference in the size of the molecules. {This can be proved using eqns. 17, 19 and 20, as with $I_{Xj} \approx I_{Xi}$ the first derivation of log $r_{i,j}$ *vs.* φ dependence is directly proportional to $(I_X - I_{H_2O} \cdot 2c_M/c_X)$, with a positive proportionality constant. $I_{H_2O} \cdot 2c_M/c_X \approx 20$ [15] and the value of the first derivation is negative for all compounds with $I_X < 20$, *i.e.*, for the vast number of compounds less polar than acetonitrile or dioxane, see Table 1.}

If the molecules of the compounds i and j are of approximately equal size, $V_{Xj} \approx V_{Xi}$, the less polar compound is retained more strongly, *i.e.*, $I_{Xj} < I_{Xi}$ and the separation selectivity decreases with increasing φ proportionally to the difference $I_{Xj} - I_{Xi}$. These predictions are in agreement with numerous experimental observations.

If one compound of the pair to be separated is at the same time larger and more polar than the other one, *i.e.*, $V_{Xj} > V_{Xi}$ and $I_{Xj} > I_{Xi}$, the difference $V_{Xj}I_{Xj} - V_{Xi}I_{Xi}$ is large and we can expect significant effects of the composition of the mobile phase on the selectivity of separation. A change in the order of elution is likely to occur at certain concentration φ_0 of the organic solvent in the mobile phase. The value of φ_0 can be calculated from eqn. 20 setting $r_{i,j} = 1$. It can be shown that φ_0 decreases with increasing $(V_{Xj}I_{Xj} - V_{Xi}I_{Xi})$ and can be either >1 or <1, while only $\varphi_0 < 1$ has physical meaning. This means that a large value of $(V_{Xj}I_{Xj} - V_{Xi}I_{Xi})$ increases the probability that φ_0 falls below 1, into the range of real concentrations. For example, benzene and anisole change their order of elution on a LiChrosorb C_{18} column in 46% methanol, 31% acetonitrile and 55% dioxane, while the values of φ_0 predicted from eqn. 20 are 43% methanol, 40% acetonitrile and 50% dioxane [23].

3. RETENTION IN HOMOLOGOUS AND OLIGOMERIC SERIES

Homologous series with methylene groups or oligomeric series with other structural repeat units are especially suitable for investigating the correlations between structure and retention behaviour. Both the size (molar volumes, V_X) and the polarity (interaction indices, I_X) of

members of such a series increase or decrease regularly with the number of repeat units, n [26–28]:

$$V_X = V_{0X} + \Delta V_X n \quad (21)$$

$$I_X = I_{0X} + \Delta I_X n \quad (22)$$

where V_{0X} and I_{0X} are the molar volume and the interaction index, respectively, of the structural residue (end groups) and ΔV_X, ΔI_X are the increments of the molar volume and of the interaction index in the series. The validity of eqns. 21 and 22 is demonstrated by Figs. 2 and 3 for four different homologous series [29]. The ΔV_X of the repeat methylene unit in these series is approximately 16 cm^3 mol^{-1}. As the methylene unit is non-polar, the polarities of the homologues decrease regularly with n and the values of ΔI_X are negative and close each to the other in various homologous series (−0.40 for the esters of aliphatic carboxylic acids, −0.36 for the esters of n-alkanols and −0.25 for n-alkylbenzenes).

The combination of eqns. 1, 21 and 22 results in the following quadratic expression for the dependence of the logarithms of the capacity factors k' on the number of the repeat structural units, n [26]:

$$\log k' = \log \beta + (\log \alpha)n + (\log \gamma)n^2 \quad (23)$$

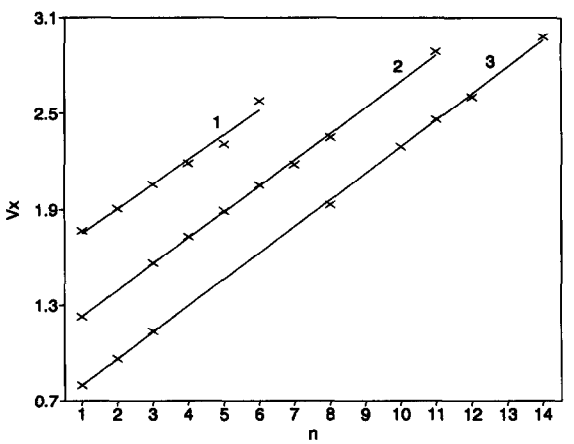

Fig. 2. Dependences of the molar volumes, V_X, on the number of methylene groups, n, in homologous series of (1) 3,5-dinitrobenzoates of n-alkanols, (2) n-alkylbenzenes and (3) methyl esters of n-alkanoic acids.

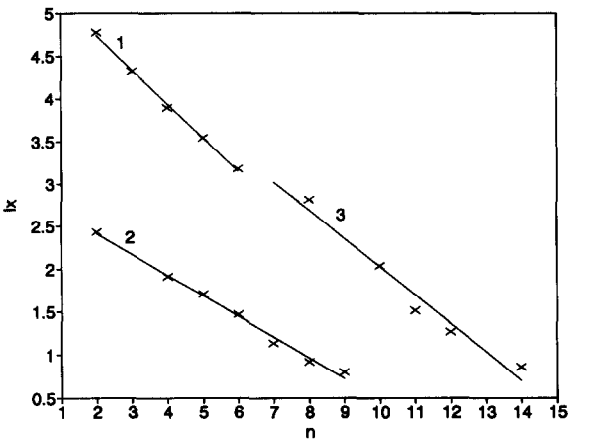

Fig. 3. Dependences of the interaction indices, I_X, on the number of methylene units, n, in homologous series of (1) 4-bromophenacyl esters of n-alkanoic acids, (2) n-alkylbenzenes and (3) methyl esters of n-alkanoic acids. Column: Silasorb C_{18}, 10 μm. Interaction indices determined as in Fig. 1.

This equation contradicts the empirical rule, according to which linear log k' *versus* n plots are expected. In fact, linear plots are most often found experimentally for various homologous and oligomeric series in different chromatographic systems, but occasionally deviations from linearity are observed if the retention is investigated over a wide range of n. These differences are usually attributed to possible changes in the conformation of the chains of repeat structural units at a certain size of the molecule. *A* closer inspection of some published data reveals that the change in the shape of the log k' *vs.* n plots is often slow rather than abrupt, which can possibly be explained by the quadratic form of eqn. 23. Anyway, the term log γ is usually small and can be neglected, so it is not considered further here. After combination of eqns. 1, 3–6, 21 and 22, eqn. 23 can be rewritten as [26,28]:

$$\log k' = \log \beta + (\log \alpha)n$$
$$= a_0 + a_1 n - (m_0 + m_1 n)\varphi + (d_0 + d_1 n)\varphi^2 \quad (24)$$

where

$$a_0 = \log \phi + c_1 V_{0X} I_{H_2O}(c_M I_{H_2O} - c_X I_{0X}) \quad (25)$$

$$m_0 = c_1 V_{0X}(2c_M I_{H_2O} - c_X I_{0X})(I_{H_2O} - I_{org}) \quad (26)$$

$$a_1 = c_1 I_{H_2O}[(c_M I_{H_2O} - c_X I_{0X})\,\Delta V_X - c_X\,\Delta I_X\,V_{0X}] \quad (27)$$

$$m_1 = c_1(I_{H_2O} - I_{org})[(2c_M I_{H_2O} - c_X I_{0X})\,\Delta V_X - c_X\,\Delta I_X\,V_{0X}] \quad (28)$$

$$d_0 = c_1 c_M V_{0X}(I_{H_2O} - I_{org})^2 \quad (29)$$

$$d_1 = c_1 c_M\,\Delta V_X(I_{H_2O} - I_{org})^2 \quad (30)$$

with $c_1 = c_M/2.31RT$.

Comparison of eqns. 3 and 24 reveals the linear dependence of the parameters a, m and d on n. The quadratic term with d_0 and d_1 is often small and can be neglected [26]:

$$\log k' = a_0 + a_1 n - (m_0 + m_1 n)\varphi \quad (31)$$

After combination of the equations

$$a = a_0 + a_1 n \quad (32)$$

and

$$m = m_0 + m_1 n \quad (33)$$

a linear correlation between the parameters m and a is predicted:

$$m = m_0 - m_1\left(\frac{a_0}{a_1}\right) + \left(\frac{m_1}{a_1}\right)a = q + pa \quad (34)$$

Good correlations between the parameters m and a were previously found experimentally for various compounds in methanol–water mobile phases, while the correlation was poor with tetrahydrofuran–water and was not found with acetonitrile–water mobile phases [30,31]. The validity of eqns. 32–34 was verified experimentally for various homologous series and good agreement was found not only in aqueous–methanolic mobile phases, but also in mobile phases containing acetonitrile, tetrahydrofuran or dioxane [26,32]. Fig. 4 illustrates the validity of eqn. 34 for homologous *n*-alkanes and *n*-alkylbenzenes in methanol–water and acetonitrile–water mobile phases [18]. For other examples, see Ref. 26.

The combination of eqns. 34 and 24 results in the following retention equation [26]:

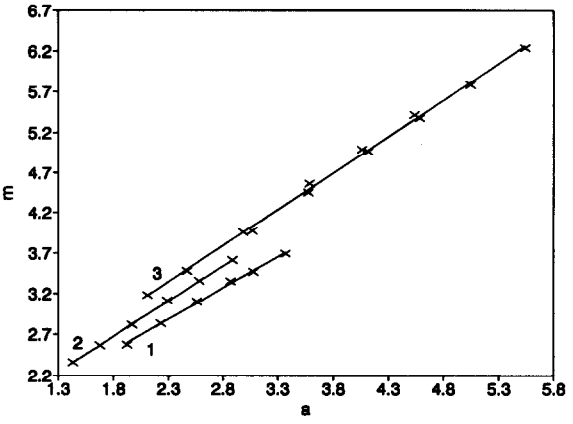

Fig. 4. Correlations between the parameters m and a of eqn. 7 for *n*-alkylbenzenes (lines 2 and 3) and *n*-alkanes (lines 1 and 3) on a Silasorb C_8, 7 μm, column (300 × 3.8 mm I.D.), in acetonitrile–water (lines 1 and 2) and methanol–water (line 3) mobile phases. Solutes: methylbenzene to *n*-hexylbenzene and *n*-heptane to *n*-dodecane.

$$\log k' = (a_0 + a_1 n)(1 - p\varphi) - q\varphi + (d_0 + d_1 n)\varphi^2 \quad (35)$$

The quadratic term in this equation can often be neglected as discussed in connection with eqns. 3, 23 and 31:

$$\log k' = (a_0 + a_1 n)(1 - p\varphi) - q\varphi \quad (36)$$

$$\log k' = (m_0 + m_1 n)\left(\frac{1}{p} - \varphi\right) - \frac{q}{p} \quad (37)$$

The slopes p of the *m versus a* plots are almost equal for various homologous series and columns in mobile phases containing a given organic solvent, but depend on the type of solvent used. The intercepts q of these plots depend on the type of homologous series and the differences between the values of q for different series are significantly lower in methanol–water mobile phases than in mobile phases containing other organic solvents, which explains the experimentally observed lack of correlation between m and a for non-homologous compounds in acetonitrile–water and tetrahydrofuran–water mobile phases [30].

It can be shown theoretically after combination of eqns. 27, 28 and 34 that if the increment $\Delta(I_X V_X) = I_{0X}\,\Delta V_X + \Delta I_X\,V_{0X}$ is approximately

constant and significantly lower than the term $c_M I_{H_2O} \Delta V_X$ (such as in homologous series with repeat methylene groups), the value of p should be constant for various series with the same repeat group and approximately equal to 2 $(1 - I_{org}/I_{H_2O})$. Consequently, p is expected to increase with decreasing polarity of the organic solvent, characterized by I_{org}. The values of p calculated from the above approximate equation using the interaction indices of solvents in Table 1 are 1.10 for methanol–water and 1.20 for dioxane–water mobile phases (the experimental values of p for various homologous series in Table 5 are lower, 0.83–0.9 for methanol–water and 0.92–1.05 for dioxane–water). The data for acetonitrile–water mobile phases are too scattered to allow meaningful comparison. For further details, see Ref. 26.

Like the constant p, the constants a_1 and m_1 do not depend significantly on the character of a homologous series and the differences in the retention between the individual homologous series are characterized mainly by different constants q and a_0 [26]. Some examples are given in Table 5.

The constant a_0 comprises the contribution of the phase ratio, ϕ, to the retention (eqn. 25) and increases with the amount and length of the bonded alkyl chains. This attribute was utilized for the characterization of the stability of various bonded stationary phases exposed to aggressive eluents by the differences in the values of a_0 before and after this treatment [33].

From eqns. 24 and 37, it can be derived that, if the dependence between log k' and φ is linear, all the log k' *versus* n plots measured for a given homologous or oligomeric series at different concentrations of the organic solvent, φ, in the mobile phase should have a common convergence point with the coordinates [27,28]

$$\log k'_c = -\frac{q}{p} = a_0 - \left(\frac{m_0}{m_1}\right) a_1 \qquad (38)$$

$$n_c = -\frac{m_0}{m_1} = \left(\frac{c_X \Delta I_X}{2 c_M I_{H_2O} - c_X I_{0X}} - \frac{\Delta V_X}{V_{0X}}\right)^{-1} \qquad (39)$$

Consequently, the series of log k' *versus* n plots corresponding to different concentrations of the organic solvent in the mobile phase have a fan-like appearance for a given homologous or oligomeric series.

Assuming linear log k' *versus* φ dependences, eqn. 31 predicts a fan-like shape with a common convergence point also for a series of the log k' *versus* φ plots for the individual homologues or oligomers. The φ coordinate of the convergence point is

$$\varphi_c = \frac{a_1}{m_1} = \frac{1}{p} \qquad (40)$$

and the k' coordinate, k'_c, is the same as for the corresponding convergence point of the set of log k' *versus* φ dependences (eqn. 38). Figs. 5 and 6 show an example of the fan-like series of log k'–n and log k'–φ dependences for higher n-alkanols on a C_{18} column in methanol–water mobile phases. A number of other examples can be found in the literature [26–28,34].

The selectivity of separation in a given homologous or oligomeric series, *i.e.*, the relative retention of two adjacent members i and j, $r_{i,j} = \alpha$ can be calculated on the basis of eqn. 24:

$$\log \alpha = a_1 - m_1 \varphi \; (+ d_1 \varphi^2) \qquad (41)$$

Neglecting the quadratic term and introducing eqns. 27 and 28 for a_1 and m_1, eqn. 41 can be rearranged [28]:

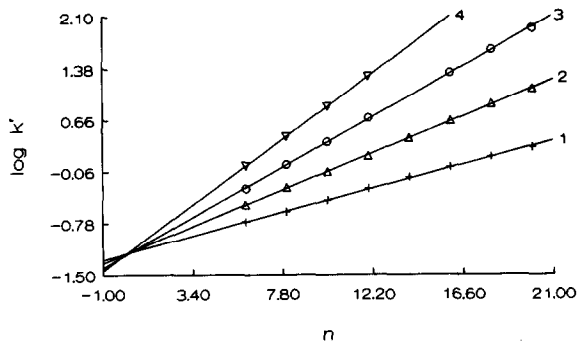

Fig. 5. Plots of the logarithms of capacity factors, k', as a function of the number of methylene groups, n, in homologous n-alkanols on a Separon SGX C_{18} column (150 × 3 mm I.D.) in (1) 100%, (2) 90%, (3) 80% and (4) 70% methanol in water.

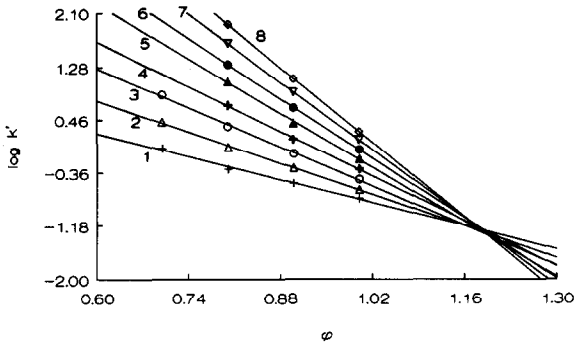

Fig. 6. Plots of the logarithms of capacity factors, k', in homologous series as a function of the concentration of methanol, φ [%(v/v)·10^{-2}], in water as the mobile phase. Column as in Fig. 5. Curves for the homologous *n*-alkanols with (1) C_6, (2) C_8, (3) C_{10}, (4) C_{12}, (5) C_{14}, (6) C_{16}, (7) C_{18} and (8) C_{20}. The common intersection point is found for a hypothetic concentration of methanol, $\varphi > 1$.

$$\log \alpha = c_1 c_X \Delta V_X \left\{ \left(2 \cdot \frac{c_M}{c_X} \cdot I_{H_2O} - Q \right) \times [I_{H_2O} - (I_{H_2O} - I_{org})\varphi] - \frac{c_M}{c_X} \cdot I_{H_2O}^2 \right\} \quad (42)$$

where

$$Q = \frac{V_{0X} \Delta I_X + I_{0X} \Delta V_X}{\Delta V_X} \quad (43)$$

As Q decreases with increasing molar volume of the repeat structural unit, ΔV_X, the separation selectivity in a homologous or oligomeric series also increases with ΔV_X and so it does with increasing polarity, I_{org}, and decreasing concentration, φ, of the organic solvent in the mobile phase, if log α is positive. These predictions agree with experimental observations (some data is given in Table 4). In addition, the combined structural parameter Q consisting of the size (ΔV_X, V_{0X}) and polarity (ΔI_X, I_{0X}) parameters of both the repeat structural unit and the end-groups is expected to affect the selectivity.

From eqn. 42 it follows that log α can be either positive or negative, *i.e.*, the members of a series can be eluted in order of either increasing or decreasing size and in a given mobile phase the order of elution is predicted to depend on the value of Q [28]. The experimentally observed behaviour usually corresponds to the first possibility. If

$$Q > \frac{c_M}{c_X} \cdot I_{H_2O} \left[2 - \frac{1}{1 - \left(1 - \frac{I_{org}}{I_{H_2O}}\right)\varphi} \right] \quad (44)$$

log α becomes negative, which means that a certain concentration of the organic solvent $\varphi = \varphi_0$ can be found where log $\alpha = 0$ and all the members of the oligomeric series are eluted in a single peak. If $\varphi > \varphi_0$, the elution order of the oligomers is opposite to the order for $\varphi < \varphi_0$ [28].

The parameter Q is low if ΔI_X is low or negative, *i.e.*, if the repeat structural unit is relatively non-polar such as the methylene group in homologous series, where $\Delta I_X < 0$ (*e.g.*, $\Delta I_X = -0.3$ in methanol–water mobile phases). ΔI_X and ΔV_X are almost independent of the type of the homologous series (see Figs. 2 and 3) and the structural parameters of the end-groups, V_{0X} and I_{0X}, often tend to compensate for the effects of each other (a bulkier end-group is likely to be less polar than a smaller group, if the differences in the polarities of the functional groups are not too great). Consequently, approximately constant values of a_1 and m_1 and of the selectivities, log α, were found for various homologous series in a given mobile phase (Tables 4 and 5) [26].

On the other hand, the parameter Q in oligomeric series becomes larger with increasing positive ΔI_X and decreasing ΔV_X, *i.e.*, for oligomeric series with relatively small and polar repeat structural units.

Eqn. 43 predicts an increase in Q also with increasing size V_{0X} and polarity I_{0X} of the end-groups. This means that we can expect significant differences in the separation selectivities between two oligomeric series with an equal repeat structural unit, if the end groups in one series are significantly bulkier than in the other. However strange the effect of the end-groups on the separation selectivity in oligomeric series may appear at a first glance, it has been observed experimentally. For example, oligoethylene glycols and ethoxylated nonylphenols have the same repeat oxyethylene unit, $-CH_2-CH_2-O-$, which is bulkier and more polar than the $-CH_2-$ group

TABLE 4

SELECTIVITIES (log α) AND SPECIFIC CONTRIBUTIONS OF THE END-GROUPS TO THE RETENTION (log β) IN SEVERAL HOMOLOGOUS AND OLIGOMERIC SERIES

Column: Silasorb C_{18}. Mobile phases: (a) methanol, (b) acetonitrile, (c) 1,4-dioxane or (d) 2-propanol in water. Homologous series: 1 = 1,2-naphthoylenebenzimidazole-6-sulphonamides of *n*-alkylamines; 2 = 3,5-dinitrobenzoates of *n*-alkanols. Oligomeric series: 3 = oligostyrenes; 4 = oligoethylene glycols; 5 = ethoxylated nonylphenols. $r_k(1)$ are the correlation coefficients of log k'–n dependences (linear eqn. 24), r and s are the intercept and the slope and $r_k(2)$ is the correlation coefficient of the log α–log β dependences (linear eqn. 45).

	Mobile phase	Series	log β	log α	$r_k(1)$	r	s	$r_k(2)$
(a)	75:25	1	−0.020	0.152	0.999			
	80:20		−0.163	0.129	0.999	−1.07	6.98	0.999
	90:10		−0.457	0.089	0.999			
(b)	70:30	1	−0.277	0.138	0.999			
	75:25		−0.332	0.124	0.999	−1.43	8.43	0.812
	80:20		−0.454	0.122	0.999			
(c)	50:50	1	−0.143	0.227	0.999			
	60:40		−0.528	0.169	0.999	−1.66	6.64	0.996
	75:25		−0.949	0.105	0.999			
(a)	70:30	2	−0.230	0.208	0.999			
	80:20		−0.479	0.154	0.999	−1.48	6.07	0.982
	90:10		−0.791	0.119	0.999			
(b)	60:40	2	−0.196	0.174	0.999			
	70:30		−0.481	0.155	0.999	−3.56	19.43	0.982
	80:20		−0.779	0.145	0.999			
(c)	50:50	2	0.215	0.266	0.999			
	60:40		−0.092	0.199	0.999	−1.13	5.11	0.999
	75:25		−0.529	0.118	0.999			
(c)	75:25	3	0.149	0.148	0.998			
	80:20		−0.012	0.102	0.999	−0.39	3.64	0.996
	85:15		−0.155	0.062	0.998			
(a)	10:90	4	−1.233	0.330	0.999			
	20:80		−1.310	0.258	0.998	−1.47	0.68	0.945
	30:70		−1.338	0.188	0.998			
(d)	45:55	5	0.762	−0.037	0.999			
	55:45		0.335	−0.039	0.999	10.24	256	0.961
	60:40		0.164	−0.039	0.999			

in a homologous series. The first oligomeric series contains two small and polar –OH end-groups, whereas the other has one –OH and one less polar, but much bulkier, nonylphenyl end group. Consequently, the value of Q is significantly larger for the ethoxylated nonylphenols than for the oligoethylene glycol series. The retentions of the two oligomeric series are very different, so that it was impossible to compare the separation selectivities for the two series in the same mobile phase on either C_{18} or C_8 columns. However, whereas ethylene glycols were eluted in order of increasing size, ethoxylated nonylphenols were almost unresolved with methanol–water mobile phases. With propanol–water mobile phases, the ethoxylated nonylphenols were eluted in order of decreasing mass [35].

In addition to the effect of higher Q, the bulkier end-group influences the condition 44 also indirectly, by significantly higher concentrations φ or lower polarities I_{org} of the organic

TABLE 5

VALUES OF THE PARAMETERS a_0, a_1, m_0, m_1, p AND q OF EQNS. 32–34 FOR VARIOUS HOMOLOGOUS AND OLIGOMERIC SERIES ON A SILASORB C_{18} COLUMN IN MOBILE PHASES CONTAINING (a) METHANOL, (b) ACETONITRILE, (c) 1,4-DIOXANE OR (d) 2-PROPANOL IN WATER

Homologous series: 1 = *n*-alkylbenzenes; 2 = *n*-alkanols; 3 = 3,5-dinitrobenzoates of *n*-alkanols; 4 = 4-bromophenacyl esters of *n*-alkanoic acids; 5 = 1,2-naphthoylenebenzimidazole-6-sulphonamides of *n*-alkylamines; 6 = 4-(N,N-dimethylamino)benzene-4'-azobenzoylamides of *n*-alkylamines; 7 = 3-*n*-alkyl-6-methyluracils; 8 = 2-alkanones; 9 = 3-*n*-alkoxycarbonyl pyrazolines; 10 = methyl esters of *n*-alkanoic acids. Oligomeric series: 11 = oligostyrenes; 12 = oligoethylene glycols; 13 = ethoxylated nonylphenols.

Mobile phase	Series	a_0	a_1	m_0	m_1	p	q
a	1	1.92	0.54	2.63	0.47	0.87	0.96
	2	−0.95	0.48	0.29	0.40	0.84	1.08
	3	1.71	0.52	2.77	0.45	0.86	1.30
	4	1.74	0.48	2.82	0.41	0.86	1.32
	5	2.17	0.47	2.92	0.42	0.90	0.96
	6	2.29	0.46	3.12	0.40	0.88	1.09
	7	−3.00	0.56	1.87	0.44	0.77	2.12
	8	−0.56	0.60	0.92	0.50	0.83	1.37
	9	−0.17	0.62	1.71	0.51	0.84	1.85
	10	0.43	0.59	0.70	0.52	0.88	1.07
b	1	1.39	0.28	2.20	0.16	0.60	1.36
	3	1.54	0.25	2.88	0.14	0.56	2.03
	4	1.42	0.26	2.61	0.16	0.64	1.65
	5	0.97	0.25	1.77	0.16	0.65	1.14
	6	1.64	0.24	2.84	0.13	0.43	0.87
	7	−0.45	0.34	2.18	0.15	0.48	2.36
	10	0.29	0.42	1.30	0.29	0.66	1.70
c	1	1.82	0.33	2.72	0.31	0.92	1.04
	3	1.70	0.55	2.98	0.58	1.05	1.19
	4	1.40	0.54	2.64	0.56	1.05	1.16
	5	1.42	0.46	3.18	0.48	1.02	1.76
d	1	1.42	0.33	2.58	0.38	1.18	0.90
c	11	2.49	0.77	3.12	0.83	1.08	0.44
a	12	−1.06	0.36	0.61	0.60	1.66	2.36
d	13	2.54	−0.03	3.97	0.01	−0.34	4.82

TABLE 6

COMPARISON OF THE VALUES OF Q WITH THE RIGHT-HAND SIDE (RHS) OF CONDITION 44 FOR OLIGOMERIC SERIES OF OLIGOETHYLENE GLYCOLS (OEG) AND OF ETHOXYLATED NONYLPHENOLS (ENP)

The values of the constants necessary for the calculations are based on the data in Table 5 in Ref. 28: $I_{H_2O} = 47$; $I_{CH_3OH} = 21.1$; $I_{C_3H_7OH} = 20.4$ (estimated); $c_M/c_X = 0.23$.

Oligomeric series	Mobile phase	RHS	Q
OEG	CH_3OH–H_2O (5:95)	10.4	8.1
OEG	CH_3OH–H_2O (30:70)	8.6	8.1
ENP	C_3H_7OH–H_2O (30:70)	8.6	13.3
ENP	C_3H_7OH–H_2O (60:40)	5.1	13.3

solvent in the mobile phase necessary to accomplish the elution in reasonable time, which diminishes the right-hand side of the condition 44 [28]. Table 6 illustrates the possibilities of prediction of the elution order in oligomeric series using the present approach. Some data from Ref. 28 were used for calculation of the values of Q and of the right-hand side of condition 44. On a C_{18} column, 5–30% methanol in water is suitable to accomplish the separation of lower oligoethylene glycols ($n = 2$–10). In these mobile phases, Q is smaller than the right-hand side of condition 44 and hence elution in order of increasing number of oxyethylene units is expected. On the other hand, 30–60% propanol in water should be used to accomplish a reasonable separation of ethoxylated nonylphenols ($n = 1$–12) and in these mobile phases Q is larger than the right-hand side of condition 44. Elution in order of decreasing n is therefore predicted. Both theoretical predictions agree with the experimental results [28].

The physical meaning of the effect of the end-groups on the separation selectivity can possibly be explained as follows. The end-groups in an oligomer form part of the environment of the repeat structural units, together with the surrounding molecules of the mobile phase and may interact with the repeat units by polar and non-polar interactions, which depend on their polarities and size. In other words, this does not mean that the Martin's rule of additivities of the group contributions to the total energy of interactions does not apply here. The contribution of a repeat group is constant (or almost so) in a given oligomeric series and chromatographic system and its value is affected not only by the character of the stationary phase and the composition of the mobile phase, but generally also by the end-group(s) in the series. The contribution of the methylene group to the retention in various homologous series in reversed-phase systems is approximately constant, which results from the small size and non-polar character of the methylene group, and this behaviour cannot be generalized and extrapolated to the series of compounds with other repeat structural units.

Neglecting the quadratic terms, eqns. 24 and 31–34 can be combined to yield:

$$\log \beta = -\frac{q}{p} + \frac{q + pa_0}{pa_1} \log \alpha = r + s \log \alpha \qquad (45)$$

This means that the separation selectivity in a homologous or oligomeric series characterized by log α should be correlated with the specific contribution of the end-groups to the retention, log β, in mobile phases with different concentrations φ of the organic solvent in water. A good correlation was found for lower values of α, slightly inferior in acetonitrile–water than in methanol–water mobile phases (Table 4). The deviations from eqn. 45 found for higher α in mobile phases with low contents of the organic solvent can probably be attributed to the non-linearity of the log $k' - \varphi$ plots at low φ. In agreement with eqn. 45, the slopes of the experimental plots are positive, but the intercepts are negative and decrease with decreasing size and increasing polarities of the end-groups (Table 5) (the parameter q increases in that direction) [27].

The selectivity in homologous series, log α, can be taken as the basis for the definition of the solvent (eluotropic) strength, ε^0, of various solvents and solvent mixtures, taking the value for pure water, log α_{H_2O}, as the reference standard of the eluotropic strength scale ($\varepsilon^0 = 0$) [36]:

$$\log \alpha_{H_2O} - \log \alpha = \Delta V_{X,CH_2} \varepsilon^0 \qquad (46)$$

$\Delta V_{X,CH_2}$ is the increment to the molar volume of a methylene group and its mean value is 16.8 ± 2.3 ml for a variety of homologous series [36]. It is interesting to note that the values of ε^0 determined using eqn. 46 for various solvents [36] are close to the values of ε^0 set equal to the mean value of m in eqn. 7 for a large variety of different compounds according to Snyder's definition [17]. The similarity of the values of ε^0 determined from the data for various more or less polar compounds to the values calculated from eqn. 46, where only non-specific selectivity for the non-polar methylene group is considered, supports the assumption that the solvophobic effect plays a major role in controlling the retention.

4. LIPOPHILIC AND POLAR INDICES

4.1. Calibration of retention using a reference homologous series

The interaction indices approach discussed in Section 2.2 requires a knowledge of the values of the phase ratio, ϕ, (the definition and the determination of the volume of stationary phase are often not straightforward) and of the molar volumes of sample solutes, V_X, which are not always known and sometimes the sample is not available in sufficient amount for the experimental determination of V_X. It is possible to overcome this inconvenience by using a homologous series of reference standard compounds for calibration of the retention and selectivity scale [37]. Using the model of interaction indices and the description of retention behaviour in homologous series presented in Sections 2 and 3, a new phenomenological approach was worked out to characterize the retention in reversed-phase chromatography [37].

The method is based on the validity of eqn. 36 in a variety of reversed-phase systems. The parameters a_1, m_1 and p in eqns. 31 and 36 have been found experimentally to be approximately constant for various homologous series and columns and to characterize the non-specific contribution to the retention, whereas the constants q, a_0 and m_0 are far more influenced by the type of homologous series and column (Table 5) [26]. This suggests that eqn. 36 with the parameters a_1 and p determined for one (calibration) homologous series can be used to describe the retention in another homologous series, if the parameters a_0 and q are determined for each individual series.

Any compound can be considered as a hypothetical member of a homologous series with $n = 0$ or $n > 0$, so that this approach can be applied to all sample solutes. In that case the values of n and of the constants a_0 and q of the sample solute are not known, but we can use the values of the parameters a_0 and a_1 of the calibration homologous series and eqns. 32 and 34 to calculate the hypothetical equivalents to the constant q, q_i, and to the number of methylene units n, n_{ce}, from the parameters a and m in eqn. 7 determined by linear regression of the log k' *vs.* φ plots for the solute measured under the same conditions as the data for the calibration homologous series:

$$n_{ce} = \frac{a - a_0}{a_1} \tag{47}$$

$$q_i = m - p(a_0 + a_1 n_{ce}) \tag{48}$$

The index n_{ce} is equivalent to the number of CH_2 groups in the calibration homologous series of the hypothetical homologue with the same lipophilic character as the sample solute so that it is a measure of the non-polar contribution to retention and can be used as a lipophilic index for the characterization of the solute. The polar contribution to retention can be characterized by the other, polar, index q_i [37].

The indices n_{ce} and q_i can be used to calculate the capacity factors of the solute in other binary mobile phases, for which the parameters a_0, a_1 and p of the calibration homologous series have been measured previously:

$$\log k' = (a_0 + a_1 n_{ce})(1 - p\varphi) - q_i\varphi \tag{49}$$

The main difference from the interaction indices approach is the use of two indices related to the calibration homologous series instead of I_X and V_X to characterize the retention of the sample in different mobile phases.

The calibration homologous series should be non-polar in order to distinguish better the specific and non-specific contributions to the retention. *n*-Alkanes are theoretically best suited to this requirement, but for practical reasons (impossibility of using UV detectors and high volatility of lower *n*-alkanes), *n*-alkylbenzenes are a better choice [37]. Because of the strong retention of higher alkylbenzenes in reversed-phase systems, the use of *n*-alkylbenzene calibration homologous series is limited to mobile phases containing 50% or more of one or two organic solvent(s). Other homologous series were tested as potential calibration standards in mobile phases with higher concentrations of water and homologous alkoxycarbonylpyrazolines or alkan-2-ones were found suitable for this purpose in mobile phases containing 25–

50% of methanol in water or 30–50% of acetonitrile in water [38].

As the parameter q depends on the column used [26,33], the parameters q_i determined on different columns can be expected to differ each from the other. Moreover, the values of the indices n_{ce} and q_i can be subject to systematic errors originating from fitting linear log k' *vs.* φ plots to the experimental data that are in fact linear only in a limited range of concentrations φ of the organic solvent in the mobile phase and to the errors in determination of the constants a_0, a_1 and p of the calibration homologous series.

It is known that the relative retention, $r_{i,j} = k'_j/k'_i$, of two solutes i and j is usually less prone to be affected by the source or by the batch of a column of a given type such as C_{18} than the absolute retention, k'_i or k'_j. Applying eqn. 36 we obtain for $r_{i,j}$ [39]:

$$\log r_{i,j} = a_1(1 - p\varphi)\,\Delta n_c - \varphi\,\Delta q \tag{50}$$

where

$$\Delta n_c = n_{ce,j} - n_{ce,i} \tag{51}$$

$$\Delta q = q_{i,j} - q_{i,i} \tag{52}$$

To diminish the errors in the determination of n_{ce} and q_i and to obtain the indices that could be better compared for different columns, the indices Δn_c and Δq as defined by eqns. 51 and 52 related to a standard compound with $n_{ce,i} = n_c(\mathrm{st})$ and $q_{i,i} = q(\mathrm{st})$ were introduced [39]. The main objective of this approach is to normalize the retention on various columns with respect to a single compound. This should lead to better agreement between the indices Δn_{ce} and Δq determined in various chromatographic systems than between the indices n_{ce} and q_i, because possible errors originating from the determination of the constant a_0 are eliminated, as this constant no longer appears in eqn. 50. The constants a_1 and p are less prone to be affected by the experimental errors, as the agreement between the values of these constants determined for various homologous series and columns is much better than that between the parameters a_0 (see Table 5). Although it is possible to use any compound with suitable retention as the reference standard, we selected the first member of the calibration homologous series (toluene of the alkylbenzene series) for the sake of convenience.

With the calibration series of *n*-alkylbenzenes and toluene as the reference standard compound, the differences between the experimental values of the indices determined on a C_{18} and on a C_8 column are more significant than the differences between the indices determined on different columns with the same length of the bonded alkyls, which means that the indices determined for a given alkylsilica column can be better transferred to another column of the same length of bonded alkyls than to other chemically bonded phases. The differences between the values of the indices determined on three different C_{18} columns were less than 0.4 for n_{ce} and q_i, 0.3 for Δn_c and 0.15 for Δq [39], which indicates a slight improvement for the relative indices, as expected. The errors in prediction of retention using this approach will be discussed in Section 4.6.

4.2. Structural correlations of lipophilic and polar indices

In accordance with the expected potentials for characterizing the lipophilicities of solutes by the indices n_{ce} or Δn_c, good agreement was found of the relative indices Δn_c with Hansch and Leo hydrophobic substituent constants, π [40] (correlation coefficient = 0.991) [39], but no correlation could be found between n_{ce} or Δn_c and Snyder's polarity indices, P' [16].

It can be shown that the relative polar index, Δq, is directly proportional to the difference between the interaction indices of the solute and of the reference standard compound after subtraction of the lipophilic contributions from the alkyl substituents, $I_{ox,i}$ and $I_{ox,s}$, respectively [39]:

$$\begin{aligned}\Delta q &\approx \frac{c_M}{2.3RT}(I_{H_2O} - I_{org}) \\ &\quad \times (c_X V_{ox,i} I_{ox,i} - c_X V_{ox,s} I_{ox,s}) \\ &\approx \text{constant} \cdot (I_{ox,i} - I_{ox,s})\end{aligned} \tag{53}$$

where $V_{0X,i} \approx V_{0X,s}$ is the molar volume of the zeroth member of the calibration homologous series. Therefore, Δq is expected to be directly proportional to the polarity of the functional group(s) in the solute.

A good correlation of the relative indices, Δq, with the Snyder's polarity indices, P', was found experimentally (correlation coeficient = 0.988) [39], which seems to confirm the suitability of the indices q_i or Δq to characterize the polarity of a solute. The relative polar indices Δq were found to increase with the polarities of the functional groups approximately in the order *n*-alkanes < polycyclic aromatic hydrocarbons < *n*-alkylbenzenes < benzene, styrene, biphenyl < halogenated benzenes < dialkyl ethers < alkyl aryl ethers, diaryl ethers < aromatic nitriles < aromatic ketones and aldehydes < aromatic amines < aromatic alcohols < phenols, alkylphenols < chlorophenols. For a given class of compounds, the Δq values were found within a relatively narrow range [39].

The dependence of the relative polar indices Δq in acetonitrile–water on Δq in methanol–water mobile phases is plotted in Fig. 7. The most apparent feature of this plot is a regular increase in Δq in the two sets of indices with increasing polarity of the sample solutes. The compounds with a common functional group are found in more or less limited regions in this graph. Some of these regions overlap and the areas in which the data for one class of compounds are spread increase with increasing polarity of the compounds.

In the absence of specific polar interactions, Δq in mobile phases containing various organic solvents should be the same and should appear on the straight line with slope = 1. The experimental data for most compounds in Fig. 7 are spaced within the interval ± 0.5 Δq units on both sides from this line (dashed limits). Because of selective polar interactions, differences in Δq values are found for mobile phases containing different organic solvents, so that the indices are shifted in the direction of stronger selective interactions, either to the methanol region below the line or to the acetonitrile region above the line.

In Fig. 7, this behaviour is observed for the

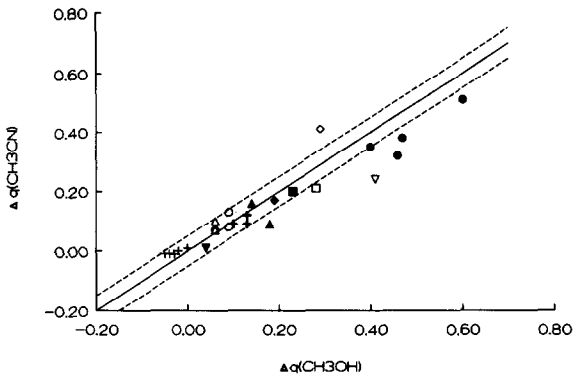

Fig. 7. Correlation between Δq_i in acetonitrile–water, $\Delta q(CH_3CN)$, and in methanol–water, $\Delta q(CH_3OH)$, mobile phases on a Silasorb C_8 column for compounds from different classes. + = alkylbenzenes; ▼ = styrene; △ = halobenzenes; ○ = alkyl aryl ethers; + = esters of carboxylic acids; ▲ = alkyl aryl ketones; □ = benzaldehyde; ■ = benzonitrile; ◇ = aniline; ▽ = benzyl alcohol; ◆ = nitrobenzene; ● = phenols.

compounds that form hydrogen bonds with –OH groups in water and lower alcohols, such as phenols, ketones and aldehydes. The points for these compounds are shifted downwards the interval limited by the dashed lines to the methanol region. The data point for aniline is shifted to the opposite direction, into the acetonitrile area.

The position of the data in the $\Delta q - \Delta q$ diagrams can be used to estimate the polarities and the presence of specific functional groups in simple organic compounds from their reversed-phase retention data.

The relative indices Δn_c and Δq are quantitative structural characteristics of sample solutes and it was found empirically that various substituents in sample solutes contribute additively to the n_{ce} and q_i (Δn_c and Δq) indices. For example, from the data published by Hammers *et al.* [41] for methylbenzenes, chlorobenzenes, chloroanilines and chlorophenols on a LiChrosorb C_{18} column in aqueous methanol, the methyl and chloro substituents and the number of substituents in adjacent positions (the *ortho* effect) were found to contribute additively to the Δn_c indices [correlation coefficients from 0.994 to 0.999 (Table 7)]. Increased contributions of a chloro substituent to Δn_c in chloroanilines and in

TABLE 7

STRUCTURAL CONTRIBUTIONS OF SUBSTITUENTS $\Delta n_{c,i}$ AND Δq_i TO THE INDICES Δn_c AND Δq FOR (SERIES A) POLYMETHYLBENZENES (1), POLYCHLOROBENZENES (2), POLYCHLOROANILINES (3) AND POLYCHLOROPHENOLS (4) AND (SERIES B) SUBSTITUTED PHENYLUREAS (5) AND SUBSTITUTED 4,6-DIAMINO-1,3,5-TRIAZINES (6)

For Series A, contributions to the indices Δn_c and Δq:

$$\Delta n_c = n_{c0} + \Delta n_{c,i} N_i + \Delta n_{c,or} N_{or}$$

$$\Delta q = q_0 + \Delta q_i N_i + \Delta q_{or} N_{or}$$

where the subscripts i and or relate to the contributions of the substituent i and of the presence of the substituents in the *ortho* position, respectively, n_{c0} and q_0 are Δn_c and Δq, respectively, of the basic molecules in the series, *i.e.*, of benzene, aniline and phenol, N_i and N_{or} are the numbers of the substituents i and of the substituents in the *ortho* position to one another, respectively; r_k are the correlation coefficients for the above equations. For Series B, for substituted phenylureas $n_{c0} = -1.4$, $q_0 = 1.60$, R_1, R_2 = substituents on nitrogen and X_1, X_2 = substituents on phenyl in positions 3 and 4, and for substituted 4,6-diamino-1,3,5-triazines $n_{c0} = -3.53$, $q_0 = 1.45$, R_1, R_2 = substituents on the amino groups and X = substituent in position 2.

Series A	Substituent	n_{c0}	$\Delta n_{c,i}$	$\Delta n_{c,or}$	r_k
1	CH_3	0.049	0.940	0.108	0.9986
2	Cl	−0.016	1.110	−0.109	0.9981
3	Cl	−1.620	1.399	0.172	0.9979
4	Cl	−1.343	1.599	−0.399	0.9944
		q_0	Δq_i	Δq_{or}	r_k
1	CH_3	0.028	−0.048	0.005	0.9592
2	Cl	0.106	−0.076	−0.010	0.9185
3	Cl	0.366	0.004	−0.074	0.8443
4	Cl	0.557	−0.116	0.060	0.7002

Series B	Substituent	Position R_1, R_2		Position X_1, X_2, X	
		$\Delta n_{ce,i}$	Δq_i	$\Delta n_{ce,i}$	Δq_i
5	CH_3	0.25	0.04	1.05	0
	i-C_3H_7			2.68	0
	C_4H_9	2.38	0.06		
	OCH_3	0.96	0.04	−0.05	0.24
	OH			−1.10	0.97
	Cl			1.33	−0.08
	Br			1.50	−0.10
	CF_3			2.39	0.06
6	CH_3	0.25	0.04		
	C_2H_5	1.25	0.06		
	i-C_3H_7	2.13	0.06		
	t-C_4H_9	3.22	0.06		
	$CH_3O(CH_2)_3$	1.69	0.13		
	Cl			1.33	−0.08
	SCH_3			2.15	−0.14

chlorophenols in comparison with chlorobenzenes can be attributed to the effects of the interactions between these substituents and the amino or phenolic groups on the lipophilicity of the solutes.

Good correlations were found also between the polar indices Δq and the number of substituents in methylbenzenes and in chlorobenzenes, where the contribution of the *ortho* effect is almost negligible. The correlation was not as good for chloroanilines, where the *ortho* effect seems to influence the values of Δq more significantly. The correlation was very poor for Δq values of chlorophenols, probably because of the interactions between the chloro substituents and the phenolic group [39].

The values of the structural contributions to the Δn_c and Δq indices of substituted benzenes are given in Table 7, together with the contributions of various substituents in phenylurea and triazine herbicides [42]. The contributions $\Delta n_{c,i}$ for the substituents on the benzene ring of substituted phenylureas agree with the contributions in methylbenzenes, chlorobenzenes and chlorophenols, *e.g.*, $\Delta n_{c,i} = 1.05$ *versus* 0.94 for a methyl group, 1.33 *versus* 1.11 for a chloro substituent and -1.29 *versus* -1.39 for a phenolic hydroxy substituent [$\Delta n_{c,i} = n_{c,0}$(chlorophenols) $- n_{c,0}$(methylbenzenes) $= -1.34 - 0.05 = -1.39$].

The polar Δq indices are only slightly or not at all influenced by the alkyl, halo or methoxy substituents and the q_i indices of all the phenylurea herbicides studied are close to one another, with the exception of hydroxymethoxuron with a phenolic hydroxyl group.

The contributions of the alkyl substituents to the Δn_c indices of substituted triazines are lower than the values for the substituents on the benzene ring and the Δq indices of the triazine herbicides are close to one another. The differences between the experimental Δn_c and the values calculated from the additive contributions are 0.13 or less and the differences between the experimental and calculated Δq indices are less than 0.06.

The experimental results support the idea of the additivities of the contributions to the Δn_c and Δq indices in the limited number of classes of compounds studied so far and more experimental data would be necessary to verify if the additivity rules for these indices are valid more generally.

4.3. Lipophilic and polar indices in homologous and oligomeric series

The additivity of the contributions to the lipophilic and polar indices is observed also in homologous and oligomeric series with regular repeat structural units. All homologous series, including the calibration one (usually *n*-alkylbenzenes), have the methylene repeat units whose contributions to the n_{ce} and q_i indices should be theoretically $n_{c,i} = 1$ and $q_I = 0$. In agreement with this prediction, the values of $n_{c,i}$ from 0.89 to 1.09 and those of q_I from -0.05 to $+0.01$ were found experimentally for six different homologous series in methanol-water and acetonitrile–water mobile phases (Table 8).

The contributions $n_{c,i}$ and q_I for oligomeric series can differ significantly from those in homologous series, according to the character of the repeat unit in the series. The experimental values of these contributions and of the contributions of the end-groups, $n_{c,E}$ and q_E, for a few oligomeric series are listed in Table 8 [28]. The contributions $n_{c,i}$ and q_I are positive for oligostyrenes and oligoethylene glycols. The contribution of the repeat unit in the oligostyrene series to the lipophilic index n_{ce} is greater than the contribution of the repeat $-CH_2-CH_2-O-$ unit in oligoethylene glycols, whereas the opposite applies for the contributions of the repeat units to the polar index q_i, in agreement with the character of the oligomeric groups.

The contribution of the end-group to the lipophilic index, $n_{c,E}$, in the oligostyrene series is equivalent to approximately four methylene units [the difference in the values of $n_{c,E}$ between oligostyrenes and *n*-alkanes is $2.0 - (-2.0) = 4.0$ (Table 8)], which corresponds to the butyl end-group in an anionically polymerized polystyrene sample. A much lower value of $n_{c,E}$ and a higher value of q_E in the oligoethylene series in comparison with *n*-alkanes is obviously due to the polar character of the hydroxyl end-groups in oligoethylene glycols.

TABLE 8

STRUCTURAL CONTRIBUTIONS OF REPEAT STRUCTURAL UNITS, $n_{c,i}$, q_I AND OF END-GROUPS, $n_{c,E}$, q_E, TO THE LIPOPHILIC AND POLAR INDICES n_{ce} AND q_i IN HOMOLOGOUS AND OLIGOMERIC SERIES

Mobile phases: (a) methanol–water, (b) acetonitrile–water, (c) 1,4-dioxane–water, (d) 2-propanol–water, (e) 2-propanol–water + 0.04 *M* CTAB. Homologous series: 1 = *n*-alkanes; 2 = 3,5-dinitrobenzoates of *n*-alkanols; 3 = 4-bromophenacyl esters of *n*-alkanoic acids; 4 = *n*-alkanols; 5 = 2-alkanones; 6 = methyl esters of *n*-alkanoic acids. Oligomeric series: 7 = oligostyrenes; 8 = oligoethylene glycols; 9 = ethoxylated nonylphenols; 10 = sulphated ethoxylated nonylphenols. r_k are correlation coefficients of the linear dependences $n_{ce} = n_{c,E} + n_{c,i}N$ and $q_i = q_E + q_I N$, where N is the number of repeat methylene or oligomeric units.

Column	Mobile phase	Series	$n_{c,E}$	$n_{c,i}$	r_k	q_E	q_I	r_k
C_8	a	1	−1.85	0.99	0.9996	1.18	−0.01	0.9000
C_8	b	1	−2.04	0.98	0.9980	1.08	−0.03	0.9454
C_{18}	a	2	−0.39	0.96		1.28	0.00	
C_{18}	a	3	−0.33	0.89		1.31	−0.01	
C_{18}	a	4	−5.35	0.89		1.11	−0.01	
C_{18}	a	5	−4.32	1.02	0.9986	1.58	−0.08	0.8890
C_{18}	a	6	−2.78	1.10		0.33	0.00	
C_{18}	c	7	2.00	2.30	0.9980	0.82	0.12	0.9964
C_{18}	a	8	−5.31	0.66	0.9494	1.56	0.28	0.9678
C_8	d	9	5.05	−0.07	0.9999	1.24	0.05	0.9999
C_{18}	d	9	3.43	−0.10	0.9889	1.00	0.05	0.9934
C_8	e	9	4.54	−0.33	0.9995	1.71	0.01	0.9160
C_{18}	e	9	3.93	−0.31	0.9983	1.48	0.02	0.9503
C_8	e	10	9.19	−0.22	0.9634	1.91	0.03	0.8726
C_{18}	e	10	9.07	−0.31	0.9972	1.92	0.01	0.9089

The contribution to the polar index, q_I, is close to zero in the series of ethoxylated nonylphenols. In contrast to the oligoethylene glycol series, a negative contribution $n_{c,i}$ of the oxyethylene unit was found, in agreement with the behaviour of this series discussed in Section 3. This means that the lipophilic character of ethoxylated nonylphenols diminishes with increasing number of oxyethylene groups, which is the reason for their elution in order of decreasing molecular mass [28]. The contribution of the end-groups to q_i and q_E is similar to that in the oligoethylene glycol series (the effect of a hydroxyl end-group), but the contribution of $n_{c,E}$ to the lipophilic index n_{ce} is much greater, because of the oxynonylphenyl structural residue (the difference between the two series corresponds to approximately ten methylene units).

A similar behaviour to that in aqueous–organic mobile phases was observed in mobile phases containing hexadecyltrimethylammonium bromide (CTAB) as the ion-pairing additive, for both ethoxylated nonylphenols and their derivatives with the hydroxyl end-group modified by sulphation. The contributions $n_{c,i}$ are negative and approximately equal for the two oligomeric series and higher than in the mobile phases without CTAB. The polar indices q_i do not depend on the number of oxyethylene units, as in propanol–water mobile phases. The greater contribution q_E of the structural residue in the sulphated oligomers in comparison with the non-sulphated series can possibly be attributed to the presence of the polar sulphuric acid ester substituent in the end-group and the greater values of $n_{c,E}$ to the formation of bulky ion pairs between the sulphated oligomers and CTAB. The analogies between the behaviours of sulphated and non-sulphated ethoxylated nonylphenols are in agreement with the ion-pair reversed-phase mechanism of separation of the sulphated ethoxylated nonylphenols in mobile phases containing CTAB.

4.4. Characterization of selectivity in binary mobile phases by lipophilic and polar indices

The selectivity of separation, $r_{i,j}$, characterized by the relative retention of two solutes, i and j, can be expressed as a function of differences in lipophilic ($\Delta n_{c,i,j} = n_{ce,j} - n_{ce,i}$) and polar ($\Delta q_{i,j} = q_{i,j} - q_{i,i}$) indices of the two solutes, introducing eqn. 49 into the definition equation for $r_{i,j}$:

$$\log r_{i,j} = \log k'_j - \log k'_i$$
$$= a_1(1 - p\varphi)\,\Delta n_{c,i,j} - \varphi\,\Delta q_{i,j}$$
$$= \log \alpha_L + \log \alpha_P \qquad (54)$$

where α_L can be understood as the lipophilic and α_P as the polar contributions to the separation selectivity, $r_{i,j} = \alpha_L \alpha_P$ [$\log \alpha_L = a_1(1 - p\varphi)\,\Delta n_{c,i,j}$; $\log \alpha_P = -\varphi\,\Delta q_{i,j}$] [39]. The constants a_1 and p relate to the calibration homologous series [n-alkylbenzenes, $a_1 = 0.6$–0.92 (Table 5); $\varphi \leq 0.8$ in most practical separations], as in eqns. 49 and 50, so that $a_1(1 - p\varphi) > 0$. The lipophilic contribution $\alpha_L > 1$ if $\Delta n_c > 0$ and is controlled by the size of the non-polar (hydrocarbon) part of the molecule; it is directly proportional to the difference between the lipophilic indices $\Delta n_{c,i,j}$ of the sample solutes. According to eqn. 54, α_L is expected to decrease with increasing concentration of organic solvent in the mobile phase, φ.

The constants a_1 and p are those of the calibration homologous series and depend on the column and on the type of organic solvent used. Both a_1 and p usually decrease with increasing polarity of the organic solvent in the mobile phase, which means that the effect of the character of the organic solvent on α_L cannot be predicted *a priori*.

The polar contribution to the separation selectivity $\log \alpha_P$ is directly proportional to the difference between the polar indices of the compounds i and j, $\Delta q_{i,j}$, and to the concentration of the organic solvent in the mobile phase, φ. For compounds with equal lipophilic indices, $\Delta n_{c,i,j} = 0$ and such pairs of solutes can be separated only on the basis of the polar

TABLE 9

EXAMPLES OF THE INFLUENCE OF THE LIPOPHILIC (α_L) AND POLAR (α_P) CONTRIBUTIONS TO THE SEPARATION SELECTIVITY, $r_{i,j}$, ON THE ORDER OF ELUTION

$r_{i,j}$(c) = calculated; $r_{i,j}$(e) = experimental values. Column: Silasorb C_8.

Mobile phase		Pair of solutes	α_L	α_P	$r_{i,j}$ (c)	$r_{i,j}$ (e)
Components	Ratio					
CH_3OH–H_2O	60:40	j = benzophenone	1.22	0.89	1.09	1.11
	70:30	i = chlorobenzene	1.16	0.88	1.02	0.99
	80:20	$\Delta n_{c,i,j} = 0.38$	1.11	0.86	0.95	0.94
	90:10	$\Delta q_{i,j} = 0.08$	1.08	0.85	0.92	0.93
CH_3CN–H_2O	55:45	j = benzophenone	1.12	0.93	1.04	1.03
	60:40	i = chlorobenzene	1.11	0.92	1.02	1.01
	70:30	$\Delta n_{c,i,j} = 0.34$	1.09	0.91	0.99	0.98
	80:20	$\Delta q_{i,j} = 0.06$	1.08	0.89	0.96	0.94
CH_3OH–H_2O	60:40	j = chlorobromuron	1.19	0.96	1.14	1.09
	70:30	i = linuron	1.15	0.95	1.09	1.11
	80:20	$\Delta n_{c,i,j} = 0.35$	1.11	0.95	1.05	1.14
	90:10	$\Delta q_{i,j} = 0.03$	1.07	0.94	1.00	0.92
CH_3CN–H_2O	55:45	j = chlorobromuron	1.13	0.93	1.05	1.08
	60:40	i = linuron	1.12	0.92	1.03	1.01
	70:30	$\Delta n_{c,i,j} = 0.35$	1.10	0.91	1.00	1.02
	80:20	$\Delta q_{i,j} = 0.06$	1.08	0.90	0.97	0.97

contribution to selectivity, $\Delta q_{i,j} \neq 0$, *i.e.*, only if the two compounds possess different polar groups.

The difference in the polar indices $\Delta q_{i,j} \approx 0$ for compounds with the same functional group(s) and the separation selectivity is controlled only by α_L. For example, this behaviour is observed for the members of a homologous series. If the functional groups in the two compounds i and j are different, the more retained compound is often both bulkier and possesses a less polar functional group, so that $\Delta n_{c,i,j} > 0$ and $\Delta q_{i,j} < 0$. Here, $\alpha_P > 1$, the separation selectivity is usually good and $r_{i,j}$ increases with decreasing concentration of the organic solvent in the mobile phase, φ, over the whole composition range of the mobile phase.

If compound j is both bulkier and contains more polar functional groups than compound i, *i.e.*, $\Delta n_{c,i,j} > 0$ and $\Delta q_{i,j} > 0$, the polar contribution to selectivity, log α_P, is of opposite sign to log α_L and diminishes the resulting selectivity log $r_{i,j}$. The value of α_L decreases with increasing concentration of the organic solvent in the mobile phase, φ, and at the same time α_P is negative and its absolute value decreases, which means that the effect of φ on the selectivity of separation is much stronger than in the previously discussed situations. The decrease in $r_{i,j}$ with increasing φ is sometimes so steep that the polar contribution may compensate for the lipophilic contribution to the selectivity of separation at a certain concentration of the organic solvent in the mobile phase, $\varphi = \varphi_0$, where $\alpha_P \alpha_L = 1$ and the two compounds are co-eluted in a single peak. For $\varphi > \varphi_0$, $\alpha_P \alpha_L < 1$, which means that the two compounds could possibly be separated, but with the reversed order of elution to that in the mobile phases with $\varphi < \varphi_0$ [39,43].

The data in Table 9 illustrate several examples of the influence of the lipophilic and polar contributions to the separation selectivity on the order of elution of two compounds, one of which (j) has higher values of both n_{ce} and q_i than the other compound (i). In these examples, the change in the order of elution was observed experimentally at the concentration of the organic solvent in the mobile phase, φ_0, differing less than 5% from the φ_0 predicted by calculation from the differences $\Delta n_{c,i,j}$ and $\Delta q_{i,j}$ using eqn. 54 [43].

4.5. Prediction of separation in ternary mobile phases

The lipophilic and polar indices can be applied also to the prediction of the capacity factors in ternary mobile phases containing organic solvents X and Y in water in concentrations φ_X, φ_Y. If the quadratic and the combined terms in eqn. 10 (last three terms on the right-hand side of this equation) can be neglected, we obtain in analogy with eqn. 49:

$$\log k' = (a_{0X} + a_{1X} n_{ce,X}) \left(\frac{1}{\varphi_X + \varphi_Y} - p_X \right) \varphi_X - q_{i,X} \varphi_X + (a_{0Y} + a_{1Y} n_{ce,Y}) \times \left(\frac{1}{\varphi_X + \varphi_Y} - p_Y \right) \varphi_Y - q_{i,Y} \varphi_Y \quad (55)$$

The subscripts X and Y denote the values of the constants and the indices in the binary mobile phases water–X and water–Y.

Using eqn. 55, the following expression for the relative retention $r_{i,j}$ of the two solutes i and j (the selectivity of separation) in ternary mobile phases is obtained [43]:

$$\log r_{i,j} = a_{1X} \Delta n_{c,i,j,X} \left(\frac{1}{\varphi_X + \varphi_Y} - p_X \right) \varphi_X - \Delta q_{i,j,X} \varphi_X + a_{1Y} \Delta n_{c,i,j,Y} \times \left(\frac{1}{\varphi_X + \varphi_Y} - p_Y \right) \varphi_Y - \Delta q_{i,j,Y} \varphi_Y \quad (56)$$

where $\Delta n_{c,i,j}$ and $\Delta q_{i,j}$ are the differences between the indices n_{ce} and q_i of the two solutes i and j. The first and third terms on the right-hand side of eqn. 56 represent the lipophilic contribution to the selectivity of separation, log α_L, and the second and fourth terms form the polar contribution, log α_P, in analogy with eqn. 54.

In homologous series, $\Delta q_i = 0$ and the separation selectivity is controlled only by the lipophilic contribution, α_L. As $\Delta n_{c,i,j}$ are approximately equal to 1 for the subsequent homologues, their relative retention, α, in ternary mobile phases

can be predicted from the values of the constants a_{1X}, a_{1Y}, p_X and p_Y of the calibration homologous series in binary mobile phases water–X and water–Y, using eqn. 56. The values of the selectivities calculated in that way, $r_{i,j}$(c), are compared in Table 10 with the experimentally determined selectivities, $r_{i,j}$(e), of three different homologous series for a C_{18} column and ternary mobile phases with various concentrations of methanol and acetonitrile in water, using *n*-alkylbenzene calibration homologous series [43]. Here, the sum of the concentrations of methanol and acetonitrile is constant and the logarithm of the selectivity $r_{i,j}$ decreases linearly with increasing concentration of acetonitrile and decreasing concentration of methanol in the mobile phase. The calculated values are in good agreement with the experimental data and the selectivities in various homologous series do not differ significantly from one another [43].

As the selectivity in homologous series is more than sufficient to separate the individual homologues in all the binary and ternary mobile phases, the influence of the composition of ternary mobile phases on the selectivity of separation of non-homologous compounds is much more interesting. Here, the differences in specific interactions are utilized to accomplish successful separations of complex sample mixtures.

Table 11 shows several application examples of eqn. 56 for the prediction of retention, k', and of separation selectivity, $r_{i,j}$, in various ternary mobile phases and compares the calculated values with the experimental data. The differences between the calculated and the experimental values are 0.06 k' units or less, with one exception [43].

Different dependences of the separation selectivity on the composition of the ternary mobile phase observed in Table 11 can be explained by the differences in the lipophilic, Δn_c, and polar, Δq, indices between the compounds i and j of the four pairs studied.

Both 1,4-dichlorobenzene and 3-chlorotoluene have low polarities and low and similar differences in the polar indices $\Delta q_{i,j}$ in acetonitrile–water and methanol–water mobile phases. As Δn_c has a greater absolute value in binary mobile phases containing methanol than in those with acetonitrile, the selectivity of separation decreases with decreasing concentration of methanol and increasing concentration of acetonitrile and because the values of both $\Delta n_{c,i,j}$ and $\Delta q_{i,j}$ are negative, reversal of the order of elution is observed between the ternary mobile phases rich in methanol and those containing higher concentrations of acetonitrile.

A similar decrease in the selectivity of separation and reversal of the order of elution occurs

TABLE 10

CALCULATED AND EXPERIMENTAL SELECTIVITIES, $r_{i,j}$, IN TERNARY MOBILE PHASES METHANOL–ACETONITRILE–WATER FOR HOMOLOGOUS SERIES

Column: Silasorb C_{18}. $r_{i,j}$(c) = values calculated from eqn. 56 with $\Delta q_{i,j}$(methanol) = $\Delta q_{i,j}$(acetonitrile) = 0 and $\Delta n_{c,i,j}$(methanol) = $\Delta n_{c,i,j}$(acetonitrile) = 1 with the constants of the calibration *n*-alkylbenzene homologous series in methanol–water ($a_1 = 0.536$, $p = 0.871$) and in acetonitrile–water ($a_1 = 0.277$, $p = 0.599$) binary mobile phases; $r_{i,j}$(e) = experimental values for *n*-alkylbenzenes (1), 3,5-dinitrobenzoates of *n*-alkanols (2) and 4-bromophenacyl esters of *n*-alkanoic acids (3).

Mobile phase (CH_3OH–CH_3CN–H_2O)	$r_{i,j}$(c)	$r_{i,j}$(e)		
		1	2	3
70: 0:30	1.60	1.63	1.61	1.58
55:15:30	1.57	1.61	1.58	1.52
40:30:30	1.53	1.58	1.54	1.49
30:40:30	1.51	1.55	1.52	1.43
15:55:30	1.48	1.51	1.48	1.46
0:70:30	1.45	1.45	1.43	1.42

TABLE 11

EXPERIMENTAL (e) AND CALCULATED (c) SEPARATION SELECTIVITIES, $r_{i,j}$ AND CAPACITY FACTORS, k', IN TERNARY MOBILE PHASES METHANOL–ACETONITRILE–WATER

Column: Silasorb C_{18}. All mobile phases contained 30% of water. Calculations from the indices $\Delta n_{c,i,j}$ and $\Delta q_{i,j}$ determined in binary mobile phases methanol–water and acetonitrile–water, respectively, using eqn. 56 and the alkylbenzene homologous series with toluene as the reference standard.

Pair of compounds	Parameter	Methanol–acetonitrile			
		70:0	55:15	15:55	0:70
j = 3-chlorotoluene	k'_j(c)	3.40	2.70	2.10	1.60
i = 1,4-dichlorobenzene	k'_j(e)	3.46	2.80	2.15	1.60
Methanol–water:	k'_i(c)	3.22	2.59	2.09	1.62
$\Delta n_{c,i,j} = -0.17$	k'_i(e)	3.28	2.76	2.15	1.63
$\Delta q_{i,j} = -0.05$	$r_{i,j}$(c)	1.06	1.04	1.00	0.99
Acetonitrile–water:	$r_{i,j}$(e)	1.05	1.02	1.00	0.98
$\Delta n_{c,i,j} = -0.10$					
$\Delta q_{i,j} = -0.02$					
j = acetophenone	k'_j(c)	0.59	0.50	0.47	0.38
i = benzonitrile	k'_j(e)	0.59	0.52	0.51	0.39
Methanol–water:	k'_i(c)	0.52	0.45	0.47	0.40
$\Delta n_{c,i,j} = -0.09$	k'_i(e)	0.51	0.46	0.51	0.40
$\Delta q_{i,j} = -0.12$	$r_{i,j}$(c)	1.14	1.11	1.00	0.95
Acetonitrile–water:	$r_{i,j}$(e)	1.16	1.13	1.08	0.97
$\Delta n_{c,i,j} = -0.34$					
$\Delta q_{i,j} = -0.06$					
j = anisole	k'_j(c)	1.14	0.94	0.81	0.65
i = nitrobenzene	k'_j(e)	1.14	0.96	0.84	0.65
Methanol–water:	k'_i(c)	0.87	0.72	0.62	0.50
$\Delta n_{c,i,j} = 0.14$	k'_i(e)	0.87	0.72	0.66	0.51
$\Delta q_{i,j} = -0.12$	$r_{i,j}$(c)	1.31	1.31	1.31	1.30
Acetonitrile–water:	$r_{i,j}$(e)	1.31	1.33	1.27	1.27
$\Delta n_{c,i,j} = 0.31$					
$\Delta q_{i,j} = -0.09$					
j = toluene	k'_j(c)	2.03	1.65	1.37	1.08
i = 3-bromonitrobenzene	k'_j(e)	2.03	1.65	1.37	1.08
Methanol–water:	k'_i(c)	1.92	1.53	1.18	0.91
$\Delta n_{c,i,j} = -0.15$	k'_i(e)	1.92	1.57	1.22	0.92
$\Delta q_{i,j} = -0.08$	$r_{i,j}$(c)	1.06	1.08	1.16	1.19
Acetonitrile–water:	$r_{i,j}$(e)	1.06	1.05	1.12	1.17
$\Delta n_{c,i,j} = -0.24$					
$\Delta q_{i,j} = -0.17$					

also for the pair acetophenone–benzonitrile, which also have negative differences $\Delta n_{c,i,j}$ and $\Delta q_{i,j}$. The difference in polar indices is more important than with the previous pair of compounds and its absolute value is greater in methanol–water than in acetonitrile–water mobile phases because of proton donor–acceptor interactions of methanol with acetophenone.

Negative $\Delta n_{c,i,j}$ and $\Delta q_{i,j}$ are observed also for the pair 3-bromonitrobenzene–toluene, but here the absolute value of the difference in the polar indices is greater in mobile phases with acetonitrile than in those containing methanol, probably because 3-bromonitrobenzene shows dipole–dipole rather than proton donor or acceptor polar interactions (toluene is non-polar). Consequent-

ly, the selectivity increases with decreasing concentration of methanol and increasing concentration of acetonitrile in the mobile phase.

The differences between the lipophilic indices $\Delta q_{i,j}$ of anisole and nitrobenzene are negative and the differences $\Delta n_{c,i,j}$ are positive. This means that both the lipophilic contribution α_L and the polar contribution α_P enhance the selectivity of separation, which is consequently significantly higher than for the other pairs of compounds in Table 11. The selectivity is almost constant over the whole composition range of the ternary mobile phases, probably because of the compensation of the effects of higher $\Delta q_{i,j}$ in methanol–water and higher $\Delta n_{c,i,j}$ in acetonitrile–water mobile phases.

4.6. Applications of lipophilic and polar indices for the characterization of separation in isocratic and gradient elution reversed-phase HPLC

The lipophilic n_{ce} or Δn_c and the polar q_i or Δq indices offer possibilities for the prediction of the retention data (k') on one column from the data measured on another, using eqn. 49 or 50. For this purpose, it is necessary to calibrate the retention scale on each column using the appropriate reference homologous series such as *n*-alkylbenzenes, to determine the constants p, a_0 and a_1.

If the values of k' are predicted on the same column used for the determination of the polar and lipophilic indices, but in a mobile phase with the concentration of the organic solvent, φ, in the mobile phase different from those used for the determination of the indices, the relative errors of the predicted values are 5% or less for most solutes in methanol–water mobile phases. For example, approximately 75% of the calculated retention volumes of fifteen phenylurea and seven triazine herbicides differed from the experimental values by less than 5% relative and 91% values differed by less than 10% relative [42].

In acetonitrile–water and tetrahydrofuran–water mobile phases, the log k' *versus* φ plots for some compounds may be curved and, consequently, the relative errors of the predicted k' values can occasionally increase up to 15–20% if the linear eqn. 49 or 50 is used [37]. The precision of the prediction can be improved by introducing an additional quadratic term with respect to φ into these equations, but this means that an additional index should be determined for each solute, which makes this approach less convenient.

The errors in prediction of the retention on a column packed with the same batch of the stationary phase, but with different dimensions to the original column, are comparable with the prediction for a single column. For example, approximately 80% of k' values of seven phenylureas and seven triazine herbicides measured on a Silasorb SPH C_{18} column (300 × 4.1 mm I.D.) differed by less than 5% relative from the values predicted using eqn. 49 and the n_{ce} and q_i indices determined on another Silasorb SPH C_{18} column (300 × 3.6 mm I.D.) [42].

The agreement between the experimental k' values measured on different columns and the predicted values usually improves if the relative indices Δn_c and Δq and eqn. 50 are used for calculations. The capacity factor of a standard reference compound, k'_{st} and its indices n_{ce}, q_i should be known for this purpose. As the first member of the calibration homologous series can be used as the reference standard, such as toluene with the *n*-alkylbenzene series, this approach does not require any additional experimental data. The relative retention $r_{i,j}$ calculated from eqn. 50 in that way is related to the reference standard and the definition equation $r_{i,j} = k'_i/k'_{st}$ is used to calculate the retention k'_i of the solute i.

In practice, this approach is limited to the columns packed with the stationary phases of equal length of the bonded alkyls, for example C_{18} columns from different manufacturers. Differences between 7 and 10% relative (with a few exceptions between 10 and 20%) were found between the predicted and experimental k' values for eight solutes with different functional groups on seven different C_{18} columns, using the above calculation approach with the *n*-alkylbenzene calibration series and toluene as the reference standard compound. However, an attempt to use this set of data for the prediction of retention on a C_8 column was unsuccessful [43].

The capacity factors of the solutes in pure water, k'_w, are useful for structure–retention relationships, but their determination by extrapolation, either linear or quadratic, of the experimental log k' *versus* φ plots is connected with very significant errors [44]. We have found good correlations between the experimentally determined log k'_w and lipophilic indices n_{ce} for nine different compounds (carbamate, phenylurea and triazine herbicides and pesticides): $n_{ce} = -5.31 + 1.72\log k'_w$; with a correlation coefficient of 0.996. When this equation was used to predict the k'_w values from the n_{ce} indices of sample solutes, a mean error of prediction of 67% was found, comparable to or less than the error in the determination of k'_w by direct extrapolation from the retention data measured in 30–80% methanol in water as the mobile phase, *i.e.*, from the parameter a in eqn. 3 or 7 [44]. This suggests the possibility of using the correlation with the n_{ce} indices to obtain rough estimates of the values of k'_w, instead of time-consuming measurement of the retention data of the solutes.

The lipophilic and polar indices were applied also to the prediction of retention in reversed-phase gradient elution chromatography. If the retention of a solute under isocratic conditions is satisfactorily described by eqn. 7, the net retention volume V'_R in gradient elution chromatography with a linear gradient of increasing concentration of the organic solvent in water can be calculated from the equation [45,46]:

$$V'_R = \frac{1}{mB_G} \log [2.31 mB_G V_M 10^{(a - mA_G)} + 1] \quad (57)$$

where a and m are the parameters of eqn. 7, V_M is the dead volume of the column and A_G and B_G are the initial concentration φ of the organic solvent at the start and the slope, respectively, of the linear gradient describing the change of the composition of the mobile phase with the volume of eluate, $\varphi = A_G + B_G V$.

Introducing the relationships between the parameters a, m and the indices n_{ce} and q_i:

$$a = a_0 + a_1 n_{ce} \quad (58)$$

$$m = q_i + p(a_0 + a_1 n_{ce}) \quad (59)$$

into eqn. 57, we obtain an expression describing the retention volume in gradient elution chromatography as a function of the indices n_{ce} and q_i. The validity of this approach was verified for eleven phenylurea herbicides on a C_{18} column using elution with linear gradients of methanol in water. The deviations of the experimental elution volumes in gradient elution chromatography from the values predicted using the lipophilic and polar indices determined under isocratic conditions were less than 10% relative [42].

It is possible in principle to combine the procedures of the optimization of the isocratic mobile phase composition or of the profile of the gradient based on the predictive calculations of retention, selectivity and resolution using eqn. 7 or 57 with the approach of lipophilic and polar indices, by using the parameters a and m calculated from the indices n_{ce} and q_i and the constants a_0, a_1 and p of the calibration homologous series (eqns. 58 and 59). The success of such predictions depends on the reliability of the predetermined data in the chromatographic system used. Gross errors can be expected in systems with significant non-linearities of the log k' *versus* φ plots and if the stationary phase affects the values of the indices by specific interactions with the solute not taken into account in theoretical considerations. It is therefore highly advisable to use the indices n_{ce} and q_i determined on the same type of column, *i.e.*, with the same length of the bonded alkyls.

An example of the application of the optimization approach based on the lipophilic and polar indices can be found in Ref. 42, where n_{ce} and q_i indices of phenylurea herbicides were used to construct a resolution map, making it possible to select simultaneously the optimum slope and the initial concentration of methanol for a linear gradient of methanol in water. Some examples given in Sections 4.4 and 4.5 illustrate the possibilities of calculations of the isocratic composition of binary or ternary mobile phases for the minimum or maximum selectivity.

5. CONCLUSIONS

The model of interaction indices offers a qualitative explanation of various effects ob-

served in reversed-phase chromatography, such as the effect of the polarity of the organic solvent in the mobile phase on possible non-linearity of the log k' *versus* φ plots, a common intersection point for a set of these plots or of the log k' *versus* n plots for a homologous or an oligomeric series, occurrence of elution in order of decreasing number of repeat structural units in some oligomeric series and the important effect of the polarity and of the size of solutes on possible changes in selectivity and reversal of the order of elution with changing composition of the mobile phase.

The interaction indices can be used for predictions of retention and selectivity in reversed-phase systems with binary or ternary mobile phases, but the precision of prediction over a wide range of mobile phase composition is improved by introducing two structural indices for each solute, characterizing the lipophilicity of the hydrocarbon part of the molecule and the polarity of the functional group(s). The structural contributions to these indices are additive in the classes of compounds studied so far. The combination of the two indices of the solutes controls the selectivity of their separation in binary and ternary mobile phases. Based on the lipophilic and polar indices, the conditions for optimum isocratic or gradient separations can be predicted.

The prediction of the retention on one column from the indices determined on another column is possible in principle, but the columns should possess the same length of the bonded alkyl chains. Limitations to this approach may originate (a) in the limited precision of the experimental data such as those caused by non-linearities of the log k' *versus* φ plots or (b) in possible specific interactions with the stationary phase such as the interactions between unreacted silanol groups in alkyl-bonded silicas and the polar solutes, mainly the basic compounds.

6. SYMBOLS

A	intercept of the calibration eqn. 13 for the scale of interaction indices
A'_0, A'_1	coefficients of the linear dependence 14 of the intercept A on the concentration of the organic solvent in the mobile phase, φ
A_G	initial concentration φ at the start of gradient elution
B	slope of the calibration eqn. 13 for the scale of interaction indices
B_G	slope of the linear gradient of the organic solvent in water in gradient elution, in $\%(v/v) \cdot 10^{-2}$/ml
B'_0, B'_1	coefficients of the linear dependence 15 of the slope B on the concentration of the organic solvent in the mobile phase, φ
I_i, I_j	interaction indices of compounds i and j, respectively
I_{H_2O}	interaction index of water
I_M	interaction index of the mobile phase
$I_{org}, I_{org,i}$	interaction indices of the organic solvent in two- or multi-component mobile phases
I_X, I_{Xi}, I_{Xj}	interaction index of the sample solute (of the sample solutes i and j, respectively)
I_{0X}	interaction index of the zeroth member of a homologous or an oligomeric series (of the end-groups)
$I_{0X,i}, I_{0X,s}$	I_{0X} of the solute and of the reference standard compound, respectively
ΔI_X	contribution to I_X of a repeat structural unit in a homologous or oligomeric series
$-\Delta G$	total free energy of retention
ΔG_{M-M}	free energy of mutual interactions between the molecules of the mobile phase
ΔG_{M-X}	free energy of interactions between the molecules of the solute and the molecules of the mobile phase
Q	term characterizing combined effects of the size and polarity of the repeat structural unit and of the end groups in an oligomeric series (eqn. 43)

R	gas constant
T	absolute temperature in Kelvins
V	volume of the eluate from the start of the elution
V_G	geometrical inner volume of the empty column
V_M	volume of the mobile phase in the column
V'_R	net retention volume
V_S	volume of the stationary phase in the column
V_X, V_{Xi}, V_{Xj}	molar volumes of the sample solute (sample solutes i and j), in $l/mol \cdot 10^{-2}$
V_{0X}	molar volume of the zeroth member of a homologous or an oligomeric series (of the end-groups)
$V_{0X,i}, V_{0X,s}$	V_{0X} of the sample solute, i, and of the reference standard, s, respectively
ΔV_X	contribution to V_X of a repeat structural unit in a homologous or an oligomeric series
$\Delta V_{X,CH_2}$	ΔV_X of the methylene group
a	log k' in pure water as the mobile phase = intercept of the log k' *vs.* φ relationships (eqn. 3, 7 or 11)
a_X, a_Y	intercepts of eqn. 7 fitted by linear regression to the experimental data in binary mobile phases water–organic solvent X or water–organic solvent Y, respectively
a_0	intercept of the linear dependence of a on n in homologous or oligomeric series (eqns. 24 and 32)
a_{0X}, a_{0Y}	a_0 in binary mobile phases water–organic solvent X or water–organic solvent Y, respectively
a_1	slope of the linear dependence of a on n in homologous or oligomeric series (eqns. 24 and 32)
a_{1X}, a_{1Y}	a_1 in binary mobile phases water–organic solvent X or water–organic solvent Y, respectively
c_M	proportionality constant between I_M and the energy of interaction for a molecule of the mobile phase
c_X	proportionality constant between I_X and the energy of interaction for a molecule of the solute
c_1	auxiliary constant, $c_1 = c_M/2.31RT$
d, d_i, d_j	second power term parameter of the log k' *vs.* φ relationships (eqn. 3) [d in mobile phase water–organic solvent i or water–mobile phase j (eqns. 9 and 10)]
d_X, d_Y	second power term proportionality constants d of eqn. 10 in binary mobile phases water–organic solvent X or water–organic solvent Y, respectively
d_0	intercept of the linear dependence of d on n in homologous or oligomeric series (eqn. 24)
d_1	slope of the linear dependence of d on n in homologous or oligomeric series (eqn. 24)
k', k'_i, k'_j	capacity factor of the sample solute (solute i or j, respectively)
k^*	specific capacity factor (eqn. 13)
k'_c	k' corresponding to the common intersection point of the log k' *vs.* n plots or of the log k' *vs.* φ plots
k'_w	experimental k' in pure water
m, m_i	parameter of the log k' *vs.* φ relationships (eqn. 3, 7 or 9) (m in mobile phase water–organic solvent i)
m_X, m_Y	slopes of eqn. 7 fitted by linear regression to the experimental data in binary mobile phases water–organic solvent X or water–organic solvent Y, respectively
m_0	intercept of the linear dependence of m on n in homologous or oligomeric series (eqns. 24 and 33)

m_1	slope of the linear dependence of m on n in homologous or oligomeric series (eqns. 24 and 33)
n	number of repeat structural units in the molecule of a homologue or an oligomer
n_c	n corresponding to the common intersection point of the log k' *vs.* n plots
$n_{ce}, n_{ce,i}, n_{ce,j}$	lipophilic structural index, equivalent to the number of methylene groups in the molecule of a hypothetical member of the calibration homologous series with the same lipophilic character as the sample solute (eqn. 47) (n_{ce} of the solute i or j, respectively)
$n_{c,i}$	contribution of a repeat structural unit to n_{ce} in a homologous or oligomeric series
$n_{c,E}$	contribution of end-groups to n_{ce} in a homologous or oligomeric series
$\Delta n_c, \Delta n_{c,i,j}$	difference between the lipophilic index of the solute and that of the reference standard (between the lipophilic indices of two solutes j and i)
$\Delta n_{c,i,j,X}, \Delta n_{c,i,j,Y}$	$\Delta n_{c,i,j}$ in binary mobile phases water–organic solvent X or water–organic solvent Y, respectively
p	slope of eqn. 34 for linear correlation between the parameters m and a in eqn. 7, applied in a homologous or oligomeric series
p_X, p_Y	p in binary mobile phases water–organic solvent X or water–organic solvent Y, respectively
q	intercept of eqn. 34 for linear correlation between the parameters m and a in eqn. 7, applied in a homologous or oligomeric series
$q_i, q_{i,i}, q_{i,j}$	polar structural index, hypothetical equivalent of the sample solute to the constant q in eqn. 35 for the calibration homologous series (eqn. 48) (q_i of the solute i or j, respectively)
q_I	contribution of a repeat structural unit to q_i in a homologous or oligomeric series
q_E	contribution of end-groups to q_i in a homologous or oligomeric series
$q_{i,X}, q_{i,Y}$	q_i in binary mobile phases water–organic solvent X or water–organic solvent Y, respectively
$\Delta q, \Delta q_{i,j}$	difference between the polar index of the solute and that of the reference standard (between the polar indices of two solutes i and j)
$\Delta q_{i,j,X}, \Delta q_{i,j,Y}$	$\Delta q_{i,j}$ in binary mobile phases water–organic solvent X or water–organic solvent Y, respectively
r	intercept of eqn. 45 describing the linear correlation between log β and log α
$r_{i,j}$	relative retention (selectivity coefficient), $r_{i,j} = k'_j/k'_i$
s	slope of eqn. 45 describing the linear correlation between log β and log α
ϕ	phase ratio in the column, $\phi = V_S/V_M$
α	relative retention $r_{i,j}$ for two adjacent members of a homologous or oligomeric series (eqn. 23)
α_{H_2O}	α with pure water as the mobile phase
α_L	lipophilic contribution to the separation selectivity (eqn. 54)
α_P	polar contribution to the separation selectivity (eqn. 54)
β	coefficient of the intercept term in the relationship between log k' and the number of repeat structural units, n, in a homologous or oligomeric series (eqn. 23)
γ	coefficient of the quadratic term in the relationship between log k' and the number of repeat structural units, n, in a homologous or oligomeric series (eqn. 23)
ε^0	eluotropic strength of a solvent
φ, φ_i	concentration of the organic solvent in aqueous–organic mobile phase (φ of the solvent i in a multi-component aqueous–organic mobile phase)
φ_X, φ_Y	φ of the organic solvent X or Y, respectively in a ternary mobile phase water–X–Y
φ_c	φ corresponding to the common intersection point of the log k' *vs.* φ plots
φ_0	φ corresponding to the co-elution of the adjacent members of a homologous or oligomeric series, $\alpha = 1$

REFERENCES

1 H. Colin, C. Eon and G. Guiochon, *J. Chromatogr.*, 122 (1976) 223.
2 H. Colin and G. Guiochon, *J. Chromatogr.*, 158 (1978) 183.
3 R.P.W. Scott, *J. Chromatogr.*, 122 (1976) 35.
4 H.D. Katz, K. Ogan and R.P.W. Scott, *J. Chromatogr.*, 352 (1986) 67.
5 P. Jandera and J. Churáček, *J. Chromatogr.*, 91 (1974) 207.
6 R. Tijssen, H.A.H. Billiet and P. Schoenmakers, *J. Chromatogr.*, 128 (1976) 65.
7 P.J. Schoenmakers, H.A.H. Billiet, R. Tijssen and L. de Galan, *J. Chromatogr.*, 149 (1978) 519.
8 P. Jandera, J. Churáček and L. Svoboda, *J. Chromatogr.*, 174 (1979) 35.
9 B.L. Karger, J.R. Gant, A. Hartkopf and P.H. Weiner, *J. Chromatogr.*, 128 (1976) 65.
10 Cs. Horváth, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.
11 Cs. Horváth and W. Melander, *J. Chromatogr. Sci.*, 15 (1977) 393.
12 D.E. Martire and R.E. Boehm, *J. Phys. Chem.*, 87 (1983) 1062.
13 R.M. McCormik and B.L. Karger, *Anal. Chem.*, 52 (1980) 2249.
14 H.E. Slaats, W. Markowski, J. Fekete and H. Poppe, *J. Chromatogr.*, 207 (1981) 299.
15 P. Jandera, H. Colin and G. Guiochon, *Anal. Chem.*, 54 (1982) 435.
16 L.R. Snyder and J.J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley-Interscience, New York, 2nd ed., 1979, p. 260.
17 L.R. Snyder, J.W. Dolan and J.R. Gant, *J. Chromatogr.*, 165 (1979) 3.
18 P. Jandera and K. Valenta, unpublished results.
19 G.E. Berendsen and L. de Galan, *J. Chromatogr.*, 196 (1980) 21.
20 H. Colin, G. Guiochon and P. Jandera, *Anal. Chem.*, 55 (1983) 442.
21 P. Jandera, J. Churáček and H. Colin, *J. Chromatogr.*, 214 (1981) 35.
22 P. Jandera, H. Colin and G. Guioichon, *Chromatographia*, 16 (1982) 132.
23 H. Colin, G. Guiochon and P. Jandera, *Chromatographia*, 17 (1983) 83.
24 P. Jandera, *Chromatographia*, 19 (1984) 101.
25 T. Tsuneyoshi, *J. Chromatogr.*, 354 (1985) 37.
26 P. Jandera, *J. Chromatogr.*, 314 (1984) 13.
27 H. Colin, A.M. Krstulovic, M.-F. Gonnord, G. Guiochon, Z. Yun and P. Jandera, *Chromatographia*, 17 (1983) 9.
28 P. Jandera, *J. Chromatogr.*, 449 (1988) 361.
29 P. Jandera and P. Kučera, unpublished results.
30 P.J. Schoenmakers, H.A.H. Billiet and L. de Galan, *J. Chromatogr.*, 185 (1979) 179.
31 T.L. Hafkenschied and M. Tomlinson, *J. Chromatogr.*, 264 (1983) 47.
32 M. Lafosse, P. Marinier, B. Joseph and M. Dreux, *J. Chromatogr.*, 623 (1992) 277.
33 M. Hetem, L. van de Ven, J. De Haan, C. Cramers, K. Albert and E. Bayer, *J. Chromatogr.*, 479 (1989) 269.
34 M. Montes, J.L. Usero, A. Del Arco, C. Izquierdo and J. Casado, *J. Chromatogr.*, 481 (1989) 97.
35 P. Jandera, *Chromatographia*, 26 (1988) 417.
36 H. Colin, G. Guiochon, Z. Yun, J.C. Diez-Masa and P. Jandera, *J. Chromatogr. Sci.*, 21 (1983) 179.
37 P. Jandera, *Chromatographia*, 19 (1984) 101.
38 P. Jandera and J. Rozkošná, *J. Chromatogr.*, 556 (1991) 145.
39 P. Jandera, *J. Chromatogr.*, 352 (1986) 91.
40 C. Hansch and A. Leo, Substituent Constants for Correlation Analysis in Chemistry and Biology, Wiley, New York, 1979.
41 W.E. Hammers, G.J. Meurs and C.L. de Ligny, *J. Chromatogr.*, 247 (1982) 1.
42 P. Jandera and M. Špaček, *J. Chromatogr.*, 366 (1986) 107.
43 P. Jandera, *J. Chromatogr.*, 352 (1986) 111.
44 P. Jandera and J. Kubát, *J. Chromatogr.*, 500 (1990) 281.
45 P. Jandera and J. Churáček, *J. Chromatogr.*, 91 (1974) 223.
46 P. Jandera, J. Churáček and L. Svoboda, *J. Chromatogr.*, 192 (1980) 37.