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METHOD FOR CHARACTERIZATION OF SELECTIVITY IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

I. DERIVATION OF THE METHOD AND VERIFICATION OF THE ASSUMPTIONS

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SUMMARY

A new method is suggested for characterization of selectivity in reversed-phase liquid chromatography. It makes use of a selectivity scale, calibrated by a reference homologous series (*n*-alkylbenzenes), and of toluene as the reference standard. The selectivity is comprised of lipophilic and polar contributions, which are characterized quantitatively by constants related to the solute structure. For certain types of compounds, these constants can be calculated from the additive increments of structural substituents. In diagrams, where the constants Δq , characterizing the polar contribution to selectivity in one binary solvent system (such as acetonitrile–water), are plotted as a function of Δq in another binary solvent system (such as methanol–water), the compounds with an equal functional group occupy narrow, limited areas and can be distinguished from compounds with other functional groups. This may offer an aid to the identification of functional groups in simple organic compounds from reversed-phase chromatographic data.

INTRODUCTION

The prediction of retention in reversed-phase liquid chromatography may be used both for the optimization of chromatographic separations and for the identification of separated compounds. The description of chromatographic behaviour in reversed-phase systems, based on the molecular connectivity concept¹, on the solvophobic theory^{2,3} or on the molecular statistical theory⁴, requires knowledge of a number of physico-chemical parameters that are often not available.

The theory based on Hildebrand solubility parameters^{5–8} and the method of interaction indices^{9,10} yield a quadratic form of the dependence of the logarithm of the capacity factor, k' , on the concentration of the organic solvent in the mobile phase, x , which often can be simplified to a linear form^{2,5–9,11–13}, but even the quadratic form is not sufficient to describe this dependence adequately over the full range of mobile phase compositions^{14,15}.

Quantitative structure-retention relationships have been studied with the aim of predicting the hydrophobic parameters useful for the determination of biological activity of various compounds¹⁶⁻²² and for the prediction of retention in reversed-phase systems²³⁻²⁹. For this purpose, the retention data have been correlated with $\log P$, the logarithm of the partition coefficient in 1-octanol-water¹⁶⁻²², with hydrophobic substituent constants^{21,23}, with molecular connectivity indices, with the shape or the volume of molecules, with Hammett's constants or with a combination of these parameters²³⁻²⁸. However, these approaches are also limited by the lack of availability of these parameters for many compounds.

Other methods for the prediction of retention in reversed-phase systems employ a scale of retention indices similar to Kováts indices, based either on alkyl aryl ketones^{30,31} or on alkan-2-ones^{32,33} as the calibration standards; these indices are valid over only a limited composition range of the mobile phases and cannot take into account eventual changes in the order of elution with changing mobile phase composition. Interaction indices, I_x , have recently been introduced for the calibration of the retention scale^{9,10}. This method allows the prediction of retention in reversed-phase systems with mobile phases containing various organic solvents, but it does not fully take into account various specific solute-solvent interactions. Even though the influence of a changing mobile phase composition on retention is reflected in the values of the calibration constants of the system, it is difficult to predict possible changes in the order of elution of compounds with changing mobile phase composition. To overcome this, another method for calibrating retention has recently been suggested, which makes use of two calibration constants for each compound³⁴. It is the goal of the present work to develop an approach by means of this method of calibrating the retention scale for the characterization of selectivity in reversed-phase liquid chromatography.

In Part I, the derivation and verification of the method is described. In Part II³⁵, the possibilities for the practical utilization of this approach will be outlined.

THEORETICAL

A simple equation has recently been derived and verified that can be used for the calculation of the capacity factors, k' , of the members of various homologous series in binary mobile phases containing an organic solvent of concentration x in water³⁶

$$\log k' = (a_0 + a_1 n_c)(1 - px) - qx \quad (1)$$

where n_c is the number of carbon atoms in the alkyl substituents of a homologous series, and a_1 and p are constants that do not depend significantly on the character of the homologous series, but on the organic solvent used as a less polar component of the mobile phase; the constants a_0 and q depend also on the type of the homologous series and on the bonded phase in the column. In certain systems, where the quadratic form of $\log k'$ vs. x plots should be considered, eqn. 1 can be modified to

$$\log k' = (a_0 + a_1 n_c)(1 - px) - qx + (d_0 + d_1 n_c)x^2 \quad (2)$$

where d_0 and d_1 are constants³⁶.

Eqns. 1 and 2 can be used for calculating the retention of a large variety of compounds, based on the calibration of the retention scale with the members of a suitably chosen homologous series, such as homologous n -alkylbenzenes. From the capacity factors of calibration homologues, measured at different mobile phase compositions, x , the constants a_0 , a_1 , p (and, if necessary, d_0 , d_1) are determined. These constants calibrate the retention scale and are considered to be generally valid also for all the other compounds. Hence, eqns. 1 or 2 can be used for calculating the constants n_{ce} and q_i for an arbitrary compound from its experimental capacity factors, measured at least at two different mobile phase compositions, x .

The constants n_{ce} and q_i are thus equivalent to n_c and q of the calibration homologous series for a given compound and can be used for calculations of k' from eqns. 1 or 2 at various compositions of the mobile phases³⁴. This approach can be simply adapted to the calibration of selectivity in reversed-phase liquid chromatography.

Selectivity is understood here as the relative retention, α , defined as the ratio of the capacity factor of the sample compound, k'_i , to the capacity factor of another compound. In order to allow a comparison of selectivity for various compounds under changing conditions, the retention of all compounds is related to a single standard compound with the capacity factor k'_s . Hence, under the given separation conditions, $\alpha = k'_i/k'_s$ and:

$$\log \alpha = \log k'_i - \log k'_s \quad (3)$$

After introducing the linear or quadratic eqns. 1 or 2 for $\log k'$ into eqn. 3, we obtain the following expressions for the selectivity

$$\log \alpha = \underbrace{a_1(1 - px)\Delta n_c}_{\log \alpha_L} - \underbrace{x\Delta q}_{\log \alpha_P} \quad (4)$$

and

$$\log \alpha = \underbrace{[a_1(1 - px) + d_1x^2]\Delta n_c}_{\log \alpha_L} - \underbrace{x\Delta q}_{\log \alpha_P} \quad (5)$$

where

$$\Delta n_c = n_{ce} - n_c(\text{st}); \quad \Delta q = q_i - q(\text{st}) \quad (6a, b)$$

and st denotes the standard compound.

If the homologous series of n -alkylbenzenes is used for the calibration of the retention scale, it is advantageous to use toluene as the standard compound for the characterization of selectivity ($n_c \approx 1$ for toluene). This selection of calibration standards makes it possible to separate selectivity into two components, lipophilic selectivity, α_L , and polar selectivity, α_P :

$$\alpha = \alpha_L \alpha_P \quad (7)$$

The lipophilic contribution, α_L , is controlled by the size of the non-polar (hydrocar-

bon) part of the molecule and is proportional to Δn_c (eqns. 4 and 5). Δn_c of a given sample compound is the equivalent to the number of carbon atoms in the alkyl chain of the calibration homologous series (*n*-alkylbenzenes), n_{ce} , related to a standard compound-member of this series (toluene) with $n_c = n_c$ (st) (eqn. 6a). α_L further depends on the character of both the column and the organic solvent used in the mobile phase, which determine the constants a , p and possibly d_1 . The value of $\log \alpha_L$ decreases with increasing concentration of the organic solvent in the mobile phase, x , either in a linear ($d_1 = 0$) or in a quadratic manner.

The polar contribution to selectivity, $\log \alpha_p = -x\Delta q$, is proportional to the difference between the constants q_i of the solute and of the standard compound and to the concentration of the organic solvent in the mobile phase. It will be shown later that the constant Δq characterizes the difference between the polarities of the functional groups in the molecules of the solute and of the standard. The value of α_p can be attributed to the interactions of the chromatographed compounds with organic solvent(s) in the mobile phase.

Theoretically, the homologous series of *n*-alkanes would be the most suitable calibration series, but because its use has practical disadvantages the use of homologous *n*-alkylbenzenes is preferred, as discussed elsewhere³⁴. *n*-Alkylbenzenes are convenient calibration standards for aromatic and heterocyclic compounds. With some aliphatic compounds that are less polar than alkylbenzenes, negative Δq values are expected, but this does not necessarily mean that the method proposed would fail for these compounds.

In the separation of selectivity into lipophilic and polar contributions it is assumed that polar interactions in the mobile phase play a far more significant rôle in the separation mechanism than a possible contribution of polar interactions in the stationary phase. The polar stationary phase interactions (such as the interactions of residual silanol groups³⁷) with sample components will influence the selectivity to some extent only if they differ significantly from the interactions with the standard. The polar interactions in the stationary phase may influence the magnitude of the parameters n_{ce} and q_i (or Δn_c , Δq) and may cause some errors in differentiating quantitatively between the polar and non-polar contributions to selectivity. These interactions are also likely to increase the importance of the quadratic term in the retention *versus* composition relationships (eqn. 5). Consequently, three parameters, Δn_c , Δq and d_1 , should be used to characterize the selectivity, in contrast to the two parameters that are sufficient if the linear relationships (eqn. 4) apply. However, it would be difficult to estimate the relative importance of the polar interactions in the stationary phase on the basis of chromatographic data alone, and this problem requires further investigation.

It is obvious that eqns. 4 and 5 can also be used to describe the selectivity (relative retention) for a pair of arbitrary compounds *i* and *j*, the separation of which is to be accomplished.

Let us investigate now the individual terms in eqns. 4 and 5 in more detail. Using the model of interaction indices, the following equation was derived for the constant q as applied to the members of an homologous series in a given system³⁶:

$$q = \frac{c_M V_{ox}}{2.3RT} [(2c_M I_{H_2O} - c_x I_{ox}) (I_{H_2O} - I_{org}) - p I_{H_2O} (c_M I_{H_2O} - c_x I_{ox})] - p \log \Phi \quad (8)$$

Φ is the phase ratio, *i.e.*, the ratio of the volumes of the stationary (V_s) and the mobile (V_M) phases in the column

$$\Phi = V_s/V_M \quad (9)$$

T is the absolute temperature, R is the gas constant, V_{ox} is the molar volume, corresponding to the residue of the molecule after an hypothetical elimination of the alkyl chain from it (zeroth member of the homologous series, $n_c = 0$), I_{H_2O} , I_{org} and I_{ox} are the interaction indices of water, the organic solvent used in the mobile phase and of the zeroth member of the calibration homologous series, c_M and c_x are auxiliary constants. After introduction of the approximate equation for the constant p^{36}

$$p \approx 2(1 - I_{org}/I_{H_2O}) \quad (10)$$

into eqn. 8, we can write:

$$\Delta q \approx \frac{c_M}{2.3RT} (I_{H_2O} - I_{org})(c_x V_{oxi} I_{oxi} - c_x V_{oxs} I_{oxs}) = \text{constant} \cdot (I_{oxi} - I_{oxs}) \quad (11)$$

Using the calibration approach, the molar volume for the zeroth member of the calibration homologous series applies to both compound *i* and standard *s* and, consequently, $V_{oxi} = V_{oxs}$. The difference, Δq , is proportional to the difference in the interaction indices of the sample solute and of the standard (after subtraction of the contributions from alkyl substituents), and it is therefore proportional to the polarity of the functional group(s) in the solute (if toluene, with a non-polar methyl group, is used as the standard). The value of Δq also depends on the temperature and on the type of the organic solvent in the mobile phase (I_{org}).

Constant p in the term for $\log \alpha_L$ depends on the type of organic solvent in the mobile phase and is not significantly influenced by the nature of the chromatographed compounds; the same holds true for constant a_1 , as has been verified elsewhere³⁶. The lipophilic term of selectivity, $\log \alpha_L$, is comprised of two contributions, the first of which does not depend on the mobile phase composition and corresponds to the selectivity in pure water as the mobile phase (as extrapolated from the experimental data in mixed aqueous-organic mobile phases). The other contribution decreases with increasing concentration of the organic solvent in the mobile phase, x . Concentration x thus influences both the lipophilic and the polar contributions to selectivity. It is interesting to note that $\log \alpha_p$ is directly proportional to x , even if the $\log k'$ vs. x plots are quadratic, and that the quadratic term contributes only to the lipophilic term, $\log \alpha_L$.

EXPERIMENTAL

The equipment used included a Model 6000A pump, an U6K injector and a Model 440 UV detector, operated at 254 nm (or a Model R-401 refractometric detector for non-UV-absorbing solutes and for the determination of column dead volumes), all from Waters Assoc. (Milford, MA, U.S.A.). The following columns, made

of stainless-steel tubing, were packed in the laboratory using a high-pressure slurry-packing technique: a, Silasorb C₁₈, 10 μm (300 \times 4.2 mm I.D.); b, Silasorb C₈, 7.5 μm (300 \times 3.8 mm I.D.). The packing materials were obtained from Lachema (Brno, Czechoslovakia). The column dead volumes, V_0 , were determined as the retention volumes of ²H₂O for each mobile phase employed. In each experiment, the flow-rate of the mobile phase was determined with a stop-watch and a graduated cylinder.

The mobile phases were prepared by mixing water (double distilled in glass apparatus with addition of potassium permanganate) with an organic solvent in the required volume ratios in the range 50–90% of the organic solvent. Methanol, acetonitrile, 1,4-dioxane and tetrahydrofuran were all of spectroscopic or pro-analysis grade (Lachema). The sample compounds were either purchased or synthesized at the Departments of Organic Chemistry and of Analytical Chemistry, University of Chemical Technology, Pardubice, Czechoslovakia.

The capacity factors were calculated from the arithmetic means of two or three experimental retention volumes, V_R , in repeated experiments:

$$k' = V_R/V_0 - 1 \quad (12)$$

To calibrate the retention scale, the capacity factors of *n*-alkylbenzenes (C₂–C₆) were measured at five different concentrations of the organic solvent used in the binary mobile phases, and the constants *a* and *m* were evaluated by using regression analysis of the experimental data, according to the equation

$$\log k' = a - mx \quad (13)$$

as described elsewhere³⁴. From constants *a* and *m*, other constants, *a*₀, *a*₁ and *p*, were calculated from the relationships

$$a = a_0 + a_1 n_{ce} \quad (14)$$

$$m = q + pa \quad (15)$$

using the data for the individual *n*-alkylbenzenes and linear regression analysis³⁵.

To calculate *n*_{ce} and *q*_i for the various non-homologous sample compounds tested, constants *a*₀, *a*₁ and *p*, determined for *n*-alkylbenzenes, were introduced into eqns. 16 and 17, together with constants *a* and *m* of eqn. 13 for each sample solute

$$n_{ce} = \frac{a - a_0}{a_1} \quad (16)$$

$$q = m - pa \quad (17)$$

and Δn_c and Δq were calculated from eqns. 6a and 6b, toluene being used as the standard compound.

RESULTS AND DISCUSSION

Evaluation of constants n_{ce} and q (Δn_c and Δq) in various systems

Constants *n*_{ce} and *q* (Δn_c and Δq) evaluated from the experimental data, may

be subject to errors from various origins. Significant errors may arise from two sources: (a) neglecting possible polar stationary phase-solute interactions and (b) evaluating the necessary constants from the experimental data. The capacity factors can be determined directly for the analytical column over only a limited range of mobile phase compositions, and constants a and m (and, if necessary, d) are evaluated by fitting linear or quadratic plots to the experimental data in only this limited region. This leads to more or less significant deviations of the parameters of the fitted lines from the "true" values of these constants. These errors influence the calculated constants, n_{ce} , q , Δn_c and Δq , and may cause differences between the values of these constants as determined for different columns or for different organic solvents in the mobile phase. To estimate the magnitude of such differences, the values of these constants determined for various columns are compared in Table I. In addition to the values determined experimentally for the Silasorb C₁₈ and C₈ columns, constants n_{ce} , q_i and Δq , evaluated from the data published earlier by Hammers *et al.*¹⁸ and by Schoenmakers *et al.*³⁹ for LiChrosorb C₁₈ columns, are also included in this table. The differences between the constants determined for the C₈ column and those for C₁₈ columns are more significant than those between the data for the individual C₁₈ columns, where the differences between the values of the constants for a given compound were less than 0.4 for n_{ce} and q_i and 0.15 for Δq . This indicates that the values of these constants, determined for a given alkylsilica gel column, could be better transferred to another column of the same chain length of bonded alkyls than to a column with other chemically bonded alkyls.

In Tables II and III, the values of constants n_{ce} , q_i and Δq , determined in binary mobile phases containing various organic solvents, are compared for the Silasorb C₈ and C₁₈ columns (provided linear dependencies of $\log k'$ on x apply). The agreement between the n_{ce} values determined in methanol-water and those in acetonitrile-water mobile phases on the Silasorb C₈ column is acceptable for most compounds tested. This may mean that the linear $\log k'$ vs. x plots are good approximations of the actual dependencies or, at least, that the deviations from linearity are similar in the two binary solvent systems tested. For the Silasorb C₁₈ column, the differences in the values of n_{ce} for some compounds in different binary mobile phases are higher; the values in tetrahydrofuran-water mobile phases, where the $\log k'$ vs. x plots are significantly curved, deviate particularly. The differences between the q_i and Δq values for different binary mobile phases can be, at least partially, attributed to selective solute-solvent interactions in the mobile phases. Therefore, it seems advisable to use the values of constants n_{ce} , q_i and Δq only for binary solvent systems containing the same organic solvent as the mobile phases used for the determination of these constants.

Verification of the polar and lipophilic contributions to selectivity

It follows from eqns. 4 and 5 that a sample component contributes to the lipophilic selectivity, α_L , by means of its carbon equivalent, Δn_c . Similarly, constant Δq characterizes the contribution of a compound to the polar selectivity, α_p . Consequently, if the separation of selectivity into lipophilic and polar contributions is justified, Δn_c and Δq for the individual compounds should be correlated to some structural parameters that characterize the lipophilicity and polarity, respectively, of the solute. This assumption was tested for several simple compounds for which the

TABLE I
COMPARISON OF n_{ce} , q_i AND Δq IN METHANOL-WATER MOBILE PHASES EVALUATED ON VARIOUS ALKYL-SILICA COLUMNS

I = Silasorb C₈; II = Silasorb C₁₈; III = LiChrosorb C₁₈ (calculated from the data of Hammers *et al.*¹⁸), IV = LiChrosorb C₁₈ (calculated from the data of Schoenmakers *et al.*³⁹). Eqns. 16, 17 and 6a, b were used for calculations.

Compounds	I			II			III			IV		
	n_{ce}	q_i	Δq									
Benzene	-	-	-	0.34	1.03	0.07	0.03	0.78	0.01	-0.03	0.52	-0.01
Toluene	1.14	1.22	0	1.10	0.95	0	1.05	0.77	0	1.06	0.53	0
Ethylbenzene	1.86	1.17	-0.05	2.02	0.96	0.01	1.91	0.78	0.01	1.97	0.52	-0.01
Biphenyl	-	-	-	-	-	-	3.36	0.86	0.09	3.23	0.54	0.01
Anisole	0.44	1.31	0.09	-0.05	0.97	0.02	0.23	0.84	0.07	-	-	-
Chlorobenzene	1.39	1.28	0.06	-	-	-	1.15	0.80	0.03	1.20	0.59	0.06
Bromobenzene	1.69	1.28	0.06	-	-	-	1.31	0.76	-0.01	-	-	-
<i>p</i> -Dichlorobenzene	-	-	-	2.19	0.99	0.04	2.17	0.76	-0.01	-	-	-
Acetophenone	-0.33	1.37	0.15	-0.84	1.13	0.18	-	-	-	-0.53	0.84	0.31
Benzophenone	1.77	1.36	0.14	-	-	-	-	-	-	1.63	0.76	0.23
Methyl benzoate	0.57	1.35	0.13	-	-	-	0.56	0.96	0.19	-	-	-
Nitrobenzene	0.27	1.41	0.19	-0.19	1.09	0.14	-0.29	0.94	0.17	-0.46	0.68	0.15
Benzonitrile	-0.32	1.45	0.23	-0.75	1.25	0.30	-0.42	1.22	0.45	-0.81	0.90	0.38
Aniline	-0.74	1.51	0.29	-	-	-	-1.68	1.02	0.25	-1.68	0.88	0.35
N,N-Dimethylaniline	-	-	-	-	-	-	0.78	0.74	-0.03	1.05	0.56	0.03
Phenol	-0.29	1.82	0.60	-	-	-	-1.32	1.19	0.42	-1.53	0.94	0.41

appropriate structural parameters can be found in the literature, using the Silasorb C₈ column and aqueous methanol as the mobile phase. The values of Δn_c and Δq found in this system are given in Table IV, together with Hansch and Leo hydrophobic substituent constants, π^{40} , and Snyder's polarity indices, P'^{41} . Jinno and Kawasaki²³ have recently published a detailed study in which they correlated the logarithms of the capacity factors of various aromatic hydrocarbons and various structural parameters, including Hansch and Leo π constants, Hammett's constants, $\log P$ (P = partition coefficient in the *n*-octanol-water system), etc. Hammers *et al.*¹⁸ reported good correlations between $\log k'$ in water (parameters a of eqn. 13) and $\log P$, and between $\log P$ and π . Therefore, the relationship between Δn_c and the hydrophobic substituent constants, π , was tested for several simple compounds in the present work and a good correlation was found, with only one outlying point for ethyl benzoate (Table IV and Fig. 1). On the other hand, very poor or no correlations

TABLE II

COMPARISON OF n_{ce} , q_i AND Δq IN METHANOL-WATER (A) AND ACETONITRILE-WATER (B) MOBILE PHASES ON A SILASORB C₈ COLUMN

Linear $\log k'$ vs. x dependencies were assumed and eqns. 16, 17 and 6a, b were used for calculations. The constants a_0 , a_1 and p were evaluated from the experimental data for *n*-alkylbenzenes: A, $a_0 = 1.542$, $a_1 = 0.501$; $p = 0.932$; B, $a_0 = 1.114$, $a_1 = 0.293$, $p = 0.864$.

Compound	n_{ce}		q_i		Δq	
	A	B	A	B	A	B
Toluene	1.14	1.11	1.22	1.12	0	0
Ethylbenzene	1.86	1.93	1.17	1.11	-0.05	-0.01
<i>n</i> -Butylbenzene	4.09	4.02	1.22	1.13	0	0.01
<i>n</i> -Hexylbenzene	5.99	6.04	1.19	1.11	-0.03	-0.01
Styrene	1.51	1.74	1.26	1.22	0.04	0.10
<i>n</i> -Hexane	4.06	3.81	1.12	0.91	-0.10	-0.21
<i>n</i> -Octane	6.09	5.98	1.10	0.87	-0.12	-0.25
<i>n</i> -Decane	7.99	7.68	1.07	0.77	-0.15	-0.35
Di- <i>n</i> -butyl ether	2.62	2.66	1.20	1.04	-0.02	-0.08
Anisole	0.44	0.51	1.31	1.25	0.09	0.13
Phenetole	1.04	1.00	1.28	1.19	0.06	0.07
<i>n</i> -Propyl phenyl ether	1.70	1.84	1.31	1.20	0.09	0.08
Bromobenzene	1.69	1.68	1.28	1.19	0.06	0.07
Chlorobenzene	1.39	1.55	1.28	1.22	0.06	0.10
α,α,α -Trichlorotoluene	3.12	3.33	1.28	1.19	0.06	0.07
Acetophenone	-0.33	-0.79	1.40	1.21	0.18	0.09
Benzophenone	1.77	1.89	1.36	1.28	0.14	0.16
Benzaldehyde	-0.18	-0.42	1.50	1.33	0.28	0.21
Phenyl acetate	-0.45	-0.68	1.35	1.24	0.13	0.12
Ethyl benzoate	1.68	1.70	1.32	1.21	0.10	0.09
Nitrobenzene	0.27	0.24	1.41	1.29	0.19	0.17
Benzonitrile	-0.32	-0.10	1.45	1.32	0.23	0.20
Aniline	-0.74	-0.03	1.51	1.53	0.29	0.41
Phenol	-0.29	-0.77	1.82	1.63	0.60	0.51
<i>o</i> -Cresol	0.12	-0.35	1.62	1.47	0.40	0.35
Linuron	1.73	1.50	1.44	1.31	0.22	0.19
Chlorbromuron	2.08	1.55	1.47	1.28	0.25	0.16

TABLE III
COMPARISON OF n_{ex} , q_i AND Δq IN METHANOL-WATER (A), ACETONITRILE-WATER (B), 1,4-DIOXANE-WATER (C) AND TETRAHYDROFURAN-WATER (D) MOBILE PHASES ON A SILASORB C₁₈ COLUMN

Linear $\log k'$ vs. x dependencies were assumed and eqns. 16, 17 and 6a, b were used for calculations; the constants a_0 , a_1 and p were evaluated from the experimental data for n -alkylbenzenes: A, $a_0 = 1.915$, $a_1 = 0.536$, $p = 0.871$; B, $a_0 = 1.393$, $a_1 = 0.277$, $p = 0.599$; C, $a_0 = 1.628$, $a_1 = 0.454$, $p = 0.978$; D, $a_0 = 1.715$, $a_1 = 0.211$, $p = 1.05$.

Compound	n_{ex}				q_i				Δq			
	A	B	C	D	A	B	C	D	A	B	C	D
Benzene	0.34	-0.02	0.18	0.00	1.03	1.35	1.12	1.15	0.07	0.03	0.04	0.02
Toluene	1.10	0.79	1.29	1.00	0.95	1.31	1.08	1.12	0	0	0	0
Anisole	-0.05	0.19	0.20	-0.50	0.97	1.47	1.27	1.24	0.02	0.16	0.18	0.12
Acetophenone	-0.84	-1.07	-0.86	-3.53	1.13	1.49	1.57	1.28	0.18	0.17	0.49	0.16
Nitrobenzene	-0.19	-0.12	-0.19	-1.10	1.09	1.56	1.45	1.37	0.14	0.25	0.37	0.25
Benzonitrile	-0.75	-0.73	-0.74	-2.09	1.25	1.55	1.56	1.32	0.30	0.24	0.48	0.19
<i>p</i> -Dichlorobenzene	2.19	1.78	2.72	2.72	0.99	1.31	1.20	2.72	0.04	0.00	0.12	0.08
<i>m</i> -Chlorotoluene	2.12	1.68	2.61	2.11	0.94	1.29	1.20	1.20	-0.01	-0.02	0.12	0.07
<i>m</i> -Bromonitrobenzene	1.25	1.03	1.77	1.52	1.03	1.48	1.48	1.35	0.08	0.17	0.39	0.22
<i>n</i> -Pentane	3.58	3.04	3.23	-	0.89	1.11	0.59	-	-0.06	-0.20	-0.50	-
<i>n</i> -Hexane	4.61	4.10	4.40	-	0.87	1.10	0.59	-	-0.08	-0.21	-0.49	-
<i>n</i> -Heptane	5.92	5.25	6.07	-	0.89	1.11	0.65	-	-0.06	-0.20	-0.43	-
<i>n</i> -Octane	6.81	6.26	6.67	-	0.86	1.11	0.60	-	-0.10	-0.20	-0.48	-
Methyl butyrate	-1.32	-1.33	-1.22	-	1.00	1.39	1.23	-	0.04	0.08	0.15	-
Methyl nonanoate	4.35	3.50	4.85	4.07	1.09	1.33	1.20	1.25	0.13	0.02	0.12	0.13
Ethyl 3,5-dinitrobenzoate	1.57	2.00	2.59	4.19	1.27	1.92	1.62	1.49	0.32	0.60	0.53	0.37
<i>n</i> -Butyl 3,5-dinitrobenzoate	3.42	3.56	4.99	7.85	1.25	1.86	1.63	1.60	0.30	0.55	0.55	0.48
<i>p</i> -Bromophenacyl propionate	1.41	1.60	1.73	-	1.27	1.73	1.54	-	0.32	0.41	0.46	-
<i>p</i> -Bromophenacyl valerate	3.30	3.33	4.16	-	1.30	1.73	1.59	-	0.34	0.42	0.51	-

TABLE IV

CORRELATIONS BETWEEN CONSTANTS Δq AND THE POLARITY INDICES P' ACCORDING TO SNYDER⁴¹ AND BETWEEN CONSTANTS Δn_c AND THE HANSCH AND LEO HYDROPHOBIC SUBSTITUENT CONSTANTS π^{40} FOR SILASORB C₈ AND METHANOL-WATER MOBILE PHASE

Δq and Δn_c values from Table II; $\Delta n_c = n_{ce} - n_{c(\text{toluene})}$. Calibration homologous series: *n*-alkylbenzenes. Reference standard: toluene. r_k = correlation coefficient.

Compound	Substituent (benzene ring)	π	Δn_c	Compound	P'	Δq
Toluene	CH ₃	0.56	0	<i>n</i> -Decane	-0.3	-0.15
Ethylbenzene	C ₂ H ₅	1.02	0.72	<i>n</i> -Hexane	0	-0.1
Bromobenzene	Br	0.86	0.55	Toluene	2.3	0
Chlorobenzene	Cl	0.71	0.25	Bromobenzene	2.7	0.06
Ethyl benzoate	COOC ₂ H ₅	0.51	0.54	Chlorobenzene	2.7	0.06
Methyl benzoate	COOCH ₃	0.01	-0.57	Phenetole	2.9	0.06
Anisole	OCH ₃	-0.02	-0.70	Anisole	3.5	0.09
Nitrobenzene	NO ₂	-0.28	-0.87	Acetophenone	4.4	0.18
Acetophenone	COCH ₃	-0.55	-1.47	Nitrobenzene	4.5	0.19
Benzonitrile	CN	-0.57	-1.46	Benzonitrile	4.6	0.23
Phenyl acetate	OCOCH ₃	-0.64	-1.59	Benzyl alcohol	5.5	0.41
Benzaldehyde	CHO	-0.65	-1.32	Aniline	6.2	0.29
Phenol	OH	-0.67	-1.43	<i>m</i> -Cresol	7.0	0.47
Acetanilide	NHCOCH ₃	-0.97	-1.86			
Benzyl alcohol	CH ₂ OH	-1.03	-1.83			
Aniline	NH ₂	-1.23	-1.88			

$$q = A' + B'P'$$

$$A' = -0.150, B' = 0.0812, r_k = 0.964^*$$

$$A' = -0.126, B' = 0.0685, r_k = 0.988^{**}$$

$$\Delta n_c = A + B\pi$$

$$A = -0.572, B = 1.281, r_k = 0.983^*$$

$$A = -0.613, B = 1.234, r_k = 0.991^{***}$$

* Regression from all the experimental values.

** Regression from all the values, except for benzyl alcohol and *m*-cresol.

*** Regression from all the values, except for ethyl benzoate.

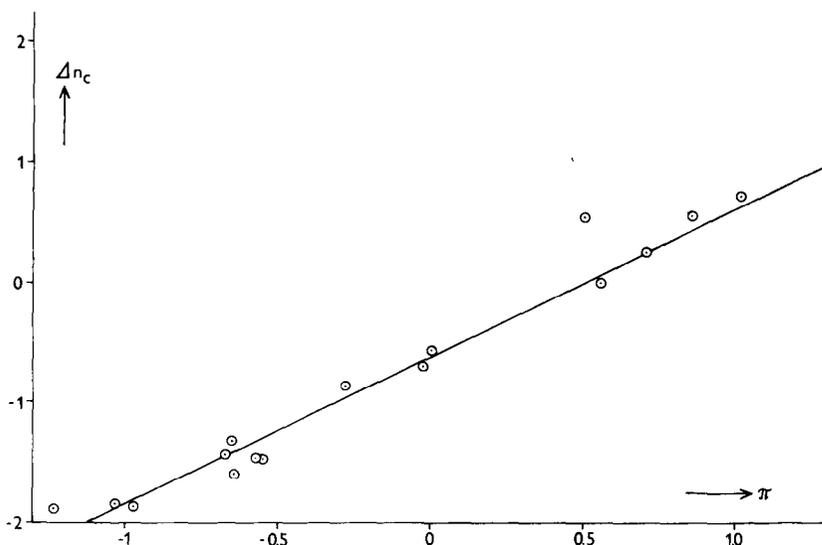


Fig. 1. Correlation between carbon equivalents, Δn_c , and Hansch and Leo hydrophobic substituent constants, π . Column: Silasorb C₈. Mobile phase: methanol-water. Compounds and values of π and Δn_c are listed in Table IV.

were found between Δn_c and P' , between Δq and Hammett's or Taft's constants and between Δq and π . The polarity indices, P' , introduced by Snyder⁴¹ to characterize solvent polarity, are a good measure of the total polarity, comprised of various selective contributions. In the present work, good correlation was found between the values of Δq and P' indices, with two outlying values for benzyl alcohol and *m*-cresol (Fig. 2). Each of these two compounds possesses an hydroxyl group, which is able to form hydrogen bonds with methanol and water. Increased interactions with the components of the mobile phase probably lead to higher values for Δq of these compounds than would be expected from the polarity indices, P' . Hence, selective interactions between the solutes and solvents used in the mobile phase can be expected to influence the values of Δq .

Relationships between constants Δn_c and Δq and the solute structure

An increase in Δq with increasing polarities of various functional groups is obvious also from the intervals in which the Δq values are found for different classes of compounds, both in methanol–water and in acetonitrile–water mobile phases (Table V). These intervals were determined from the experimental data with Silasorb

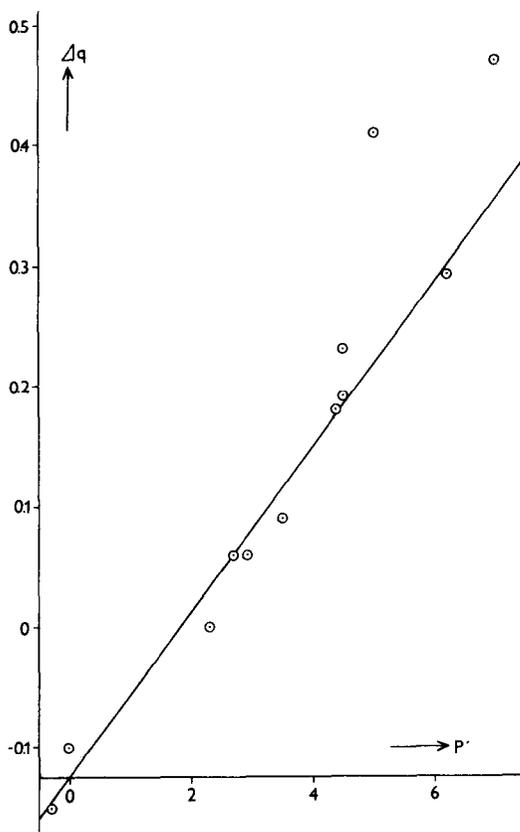


Fig. 2. Correlation between the constants Δq and Snyder's polarity indices, P' . Conditions as in Fig. 1. Compounds and values of P' and Δq are listed in Table IV.

TABLE V

INTERVALS OF Δq VALUES FOR DIFFERENT CLASSES OF COMPOUNDS, ESTIMATED FROM THE EXPERIMENTAL DATA FOR VARIOUS C_{18} and C_8 COLUMNS

Standard compound: toluene.

Class of compounds	Δq	
	Methanol-water	Acetonitrile-water
<i>n</i> -Alkanes	-0.09 to -0.15	-0.48 to -0.8
Polycyclic aromatic hydrocarbons	-0.26 to 0.25	
Di- to hexaalkylbenzenes	-0.22 to -0.05	
Monoalkylbenzenes	-0.05 to 0.04	-0.06 to 0.04
Benzene, biphenyl, bibenzyl, styrene	0.01 to 0.09	≈ 0.05 to 0.2
Bromobenzene	-0.01 to 0.06	≈ 0.03
Chlorobenzenes mono-	0.02 to 0.06	≈ -0.04
di-	-0.04 to 0.04	
tri-	-0.22 to -0.06	
tetra-	-0.24 to -0.17	
penta-	≈ -0.33	
hexa-	≈ -0.47	
Benzylchloride, benzotrithloride, DDT, dieldrin	0.06 to 0.19	≈ -0.23 to -0.08
Fluorobenzene	≈ 0.08	
Dialkyl ethers	-0.07 to -0.02	≈ -0.1
Alkyl aryl ethers	0.02 to 0.27	-0.08 to 0.06
Diaryl ethers	0.02 to 0.27	-0.08 to 0.06
Nitro- and dinitrobenzenes, bromonitrobenzene	0.08 to 0.19	0.12 to 0.24
Aliphatic esters	0.02 to 0.15	-1 to -0.72
Aromatic esters	0.1 to 0.2	0.03 to 0.31
3,5-Dinitrobenzoates, <i>p</i> -bromophenacyl esters	0.29 to 0.36	0.14 to 0.44
Aromatic nitriles	0.23 to 0.45	0.03 to 0.14
Phenylurea herbicides	≈ 0.2 to 0.3	≈ 0.14 to 0.44
Aromatic aldehydes	0.22 to 0.44	≈ 0.3 to 0.4
Aromatic ketones	0.14 to 0.48	0.06 to 0.35
Aniline, methylaniline, nitroaniline, quinoline	0.25 to 0.35	
Chloroanilines mono-	0.34 to 0.43	
di-	0.18 to 0.42	
tri-	0.13 to 0.32	
tetra-	0.02 to 0.14	
penta-	≈ -0.09	
N,N-Dimethylaniline	-0.03 to 0.03	
Aromatic alcohols	0.34 to 0.59	> 1
Phenols, alkylphenols, nitrophenols	0.25 to 0.60	0.62 to 0.84
Chlorophenols mono-	≈ 0.47 to 0.51	
di-	0.30 to 0.56	
tri-	0.24 to 0.35	

C_{18} and C_8 columns and from the data published earlier by Hammers *et al.*¹⁸ and by Schoenmakers *et al.*³⁹ for LiChrosorb C_{18} columns. The intervals in this table are only rough estimates, because they were determined for a limited number of compounds from each class (sometimes as few as two) and different lengths of the bonded alkyls in the stationary phase. On the other hand, Δq values were found within a relatively narrow range for such classes of compounds, where a greater number of compounds with the same functional group were tested (Tables I-III, VI).

To investigate in detail the relationships between the structure of various compounds belonging to a certain class and constants Δn_c and Δq , the experimental capacity factors of Hammers *et al.*¹⁸ for methylbenzenes, chlorobenzenes, chloroanilines and chlorophenols on LiChrosorb C₁₈ in aqueous methanol as the mobile phase were used. Constants n_{ce} and Δq , calculated from these data, are given in Table VI. For each class of compounds, a double linear regression analysis was used to determine the correlation between the n_{ce} and Δq constants, on one hand, and either (1) the number of the methyl and chloro substituents on the benzene ring or (2) the number of the neighbouring substituents in *ortho* positions, on the other hand. The regression equations and the correlation coefficients are also given in Table VI. The contributions of methyl groups, of chloro substituents and of the *ortho* effect to Δn_c were found to be additive for all four classes of compounds (correlation coefficients of 0.994–0.999): $\Delta n_{c(i)} = 0.94$ for a methyl and 1.11 for a chloro substituent; the contribution of the *ortho* effect, $\Delta n_{c(0)} = -0.11$ for these two groups in methylbenzenes and chlorobenzenes; $\Delta n_{c(i)}(\text{Cl}) = 1.4$ for chloroanilines and 1.6 for chlorophenols. The increased values with respect to chlorobenzenes can be attributed to the interactions between the chloro substituents and the amino or phenolic groups. These interactions are probably responsible for increased values for the contribution of the *ortho* effect ($\Delta n_{c(0)} = -0.17$ for chloroanilines and -0.40 for chlorophenols) and for a poorer correlation in the case of chlorophenols.

Good correlations were also found between constants Δq and the number of methyl ($\Delta q_{(i)} = -0.05$) and chloro ($\Delta q_{(i)} = -0.08$) substituents for methylbenzenes and chlorobenzenes. The influence of the *ortho* effect on Δq is practically negligible for these two classes of compounds. A significantly poorer correlation was found for chloroanilines, where the *ortho* effect seems to influence the Δq values more significantly than does the number of chloro substituents. The correlation for Δq values of chlorophenols is very poor and does not allow any conclusions to be drawn (probably because of the interactions between the chloro substituents and the phenolic group).

These correlations (with the exception of that for Δq of chlorophenols) can be used to describe n_{ce} and Δq values of the investigated classes of compounds with an acceptable precision, as demonstrated in Table VI. If the influence of the interactions between the individual functional groups in the molecules of a solute is of only limited importance, the structural contributions to n_{ce} (Δn_c) are additive to a first approximation.

For example, the difference in n_{ce} between bromobenzene and chlorobenzene is 0.3 in aqueous methanol for a C₈ column, and the difference between n_{ce} of chlorbromuron and linuron (one chlorine atom in linuron is replaced by one bromine atom in chlorbromuron) is 0.35 (Table II). It is interesting that the contribution of one methylene or methyl group in the alkyl aryl ether series does not correspond to $\Delta n_{c(i)} = 1$, but to 0.62. The value of n_{ce} for benzene corresponds to n_{ce} for ethane (as extrapolated from the n_{ce} values of higher *n*-alkanes) and the contribution of the ether oxygen to n_{ce} can be estimated as the difference between the n_{ce} of anisole (minus O-methyl) and toluene (minus C-methyl), *i.e.*, $0.44 - 0.62 - (1.14 - 1) = -0.32$. Then, the value of n_{ce} for di-*n*-butyl ether can be estimated as $-2 + 8 \cdot 0.62 - 0.32 = 2.64$ (the experimental value is 2.62).

From eqn. 11 it follows that the plots of Δq values in one binary solvent system, *e.g.*, acetonitrile–water *vs.* the Δq values in another binary solvent system, *e.g.*,

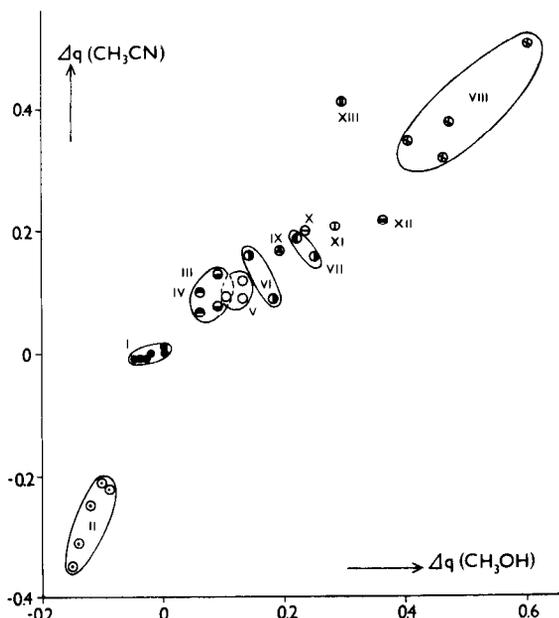


Fig. 3. Dependence of Δq in acetonitrile–water mobile phases on Δq in methanol–water mobile phases for a Silasorb C_8 column. Classes of compounds and individual compounds: I = alkylbenzenes; II = alkanes; III = bromo- and chlorobenzenes; IV = alkyl aryl ethers; V = esters of aromatic carboxylic acids and of aromatic alcohols; VI = alkyl aryl ketones, diaryl ketones; VII = halogenated phenylureas; VIII = phenol, alkylphenols; IX = nitrobenzene; X = benzonitrile; XI = benzaldehyde; XII = *n*-butyl-*N*-phenyl carbamate; XIII = aniline.

methanol–water should be described by a straight line with an intercept equal to zero, assuming the absence of specific interactions between the solutes and the organic solvents in the mobile phase. These interactions would cause deviations of the points from the line in the direction of a stronger specific interaction. Fig. 3 shows the plot of Δq values in acetonitrile–water mobile phases as a function of Δq values in methanol–water mobile phases, determined for the Silasorb C_8 column. A shift to higher Δq values with increasing polarities of the functional groups in the solutes is obvious, and the deviations of the individual points from the linear dependence because of selective solute–solvent interactions are also apparent. The points for the compounds with an identical functional group (compounds that differ only in the number or chain length of alkyls) are close together in clearly distinguishable areas (with some overlaps). Hence, diagrams such as Fig. 3 for two (or more) binary mobile phases may be useful for the identification of the functional groups in sample components on the basis of chromatographic data in reversed-phase systems.

Fig. 4 shows a similar diagram for the Silasorb C_{18} column, in which the Δq values determined from the experimental data of Schoenmakers *et al.*³⁹ for a Li-Chrosorb C_{18} column are also included. A similar dependence is obtained as in Fig. 3, but the areas for the individual classes of compounds are more or less shifted in comparison to Fig. 3. (There are also other classes of compounds included in Fig. 4, for which data were not available on the C_8 column.) An upward shift to higher

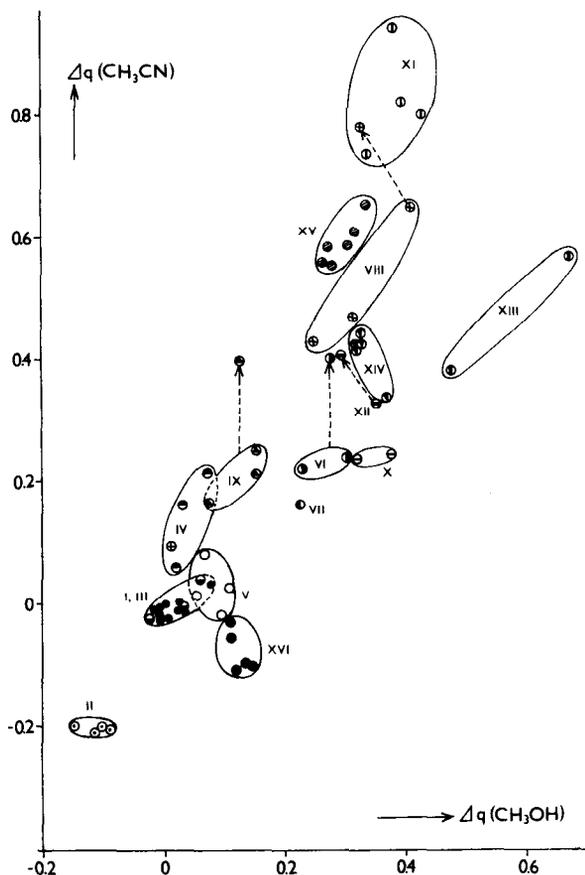


Fig. 4. Dependence as in Fig. 3 but for a Silasorb C_{18} column. Classes of compounds and individual compounds: I = alkylbenzenes; II = alkanes; III = bromo- and chlorobenzenes; IV = alkyl aryl ethers, diaryl ethers; V = esters of aliphatic carboxylic acids; VI = alkyl aryl ketones, diaryl ketones, *p*-nitroacetophenone*; VII = benzaldehyde; VIII = phenol, alkylphenols, *m*-nitrophenol*; IX = mononitroalkylbenzenes and mononitrochlorobenzenes, *m*-dinitrobenzene*; X = benzonitrile; XI = alkyl aryl alcohols; XII = aniline, *o*-nitroaniline*; XIII = diesters of aromatic carboxylic acids (phthalates); XIV = *p*-bromophenacyl esters of aliphatic carboxylic acids; XV = 3,5-dinitrobenzoates of aliphatic alcohols; XVI = 1,2-naphthoylebenzimidazole-6-sulphonamides of *n*-alkylamines. The compounds marked with an asterisk correspond to the shifted points in the diagram (nitro compounds). In this figure, the data calculated from the capacity factors on a LiChrosorb C_{18} column, published by Schoenmakers *et al.*³⁹, are also included.

Δq (acetonitrile) values for nitro compounds can be clearly recognised [*m*-dinitrobenzene with respect to benzene, *p*-nitroacetophenone with respect to acetophenone, *m*-nitrophenol with respect to phenol and *o*-nitroaniline with respect to aniline; relatively high Δq (acetonitrile) values for 3,5-dinitrobenzoates], which demonstrates the influence of selective interactions of the nitro group with acetonitrile in the mobile phase.

It can be also seen that the dispersion of the points for compounds belonging to a certain class increases with increasing values of Δq , and the higher the Δq values the larger are the areas for the individual classes of compounds.

CONCLUSIONS

The separation of selectivity into lipophilic and polar contributions is supported by the experimental correlations with lipophilic and polar structural parameters, by the fact that the Δq values are relatively independent of the number and length of the alkyls in the solute molecules and that it is possible to calculate Δn_c values from additive increments. Some exceptions from these general trends should be accounted for. For example, the interactions between various functional groups in a solute molecule may impair or vitiate additive predictions of Δn_c or of Δq values. On the other hand, specific solvent-solute interactions can be reflected in Δq values, in contrast to interaction indices.

The Δq - Δq diagrams offer an interesting aid to the identification of functional groups in simple organic compounds from reversed-phase chromatographic data.

The Δq and Δn_c data, determined for one column, are in approximate agreement with the data for the same compound, determined on another column with equal bonded alkyls (some deviations can be expected because of possible polar interactions in the stationary phase). However, one should be careful when attempting to transfer these data to columns with bonded alkyls of a different length, or to other mobile phase compositions. In the present work, mobile phases containing 50-90% of an organic solvent were investigated. In this composition range, calculations of Δn_c and Δq values can be based on the assumption of linear relationships for $\log k'$ vs. mobile phase composition (eqn. 13). However, these relationships are not linear if the complete binary mobile phase composition range is considered (from 0 to 100% of the organic solvent), and it is probable that more or less different values for constants a and m of eqn. 13 (and, consequently, different Δn_c and Δq values) will be found when the lines are fitted to experimental data for mobile phases containing less than 50% of organic solvent, or if non-linear regression is used to fit the curves. This is the subject of further investigations.

The present method for the characterization of selectivity makes it possible to differentiate between a polar and a non-polar contribution to selectivity. This may permit the use of the polar constants, Δq , as an aid in the identification of functional groups in simple organic compounds. On the other hand, using two parameters to characterize selectivity instead of a single one offers a means for predicting retention over a relatively large range of mobile phase compositions. The possibilities of the present method to predict selectivity (relative retention) and absolute retention in binary and ternary mobile phases will be treated in Part II^{3,5}. The use of Δq and Δn_c constants, once determined, for calculations of selectivity and retention on other reversed-phase columns will be also considered there.

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