

Review

Functional group contributions to the retention of analytes in reversed-phase high-performance liquid chromatography

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ABSTRACT

The application of functional group contributions (τ values) to the understanding of the retention of analytes in high-performance liquid chromatography is examined. Group contributions from a range of analytes are compared and their relationship to Hansch substituent constants (π values) determined. The effects of interactions between groups in polyfunctional analytes and of changes in the organic modifier and composition of the mobile phase are studied and the influence of different stationary phase materials compared. The potential of functional group contribution values for the prediction of relative and absolute retention is examined.

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1. INTRODUCTION

There has long been an interest in exploring the relationship between the chemical structure and physical properties of an analyte and its behaviour in reversed-phase high-performance liquid chromatography (HPLC). A comprehension of the effects of these properties on the interactions between an analyte and the stationary and liquid phases should enable the analyst to predict the separation conditions to achieve a desired retention, to select conditions for the optimum separation of a mixture of analytes, or to propose changes in conditions that should enhance a separation.

This work has been approached from two principal directions. In the first, a range of physical and structural properties, such as size, planarity, connectivity indices, dipole moment, pK_a and partition coefficients ($\log P$), have been correlated by direct comparison and multivariate analysis with measured retention times. These quantitative structure–retention relationships (QSRRs) enable the more significant parameters and their relative contributions to be determined. These QSRR methods have been the subject of extensive studies in recent years and are described in a number of reviews and books [1–5]. The factors which influence retention have recently been also discussed by Hanai [6].

Numerous systems have been studied and the correlations are often excellent. However, the resulting equations can have limited applicability, particularly those based on the more complex topological indices. They are often valid only for the closely related group of compounds from which they have been derived and are inapplicable as general prediction methods. In many cases a small change in the original data set would probably cause large differences in the significance of the different factors. There is sometimes little relation between the parameters being correlated and properties that might be intrinsically expected to bear a relationship to retention. Consequently, although the results show high statistical correlations they often contribute little to the understanding of chromatographic retention or retention prediction. Sometimes the study seems to be an exercise in

chemometrics in which every possible parameter has been included to determine, which if any, will give an acceptable correlation.

Of more widespread interest has been the numerical relationship between octanol–water partition coefficients ($\log P$) and retention because of the importance of the former in quantitative structure–activity relationship (QSAR) studies in pharmaceutical chemistry. For groups of related compounds there is often a close relationship between the measured or calculated $\log P$ values (based on their Hansch π or Rekker f functional group contributions) and retention. However, the relationships for different groups of analytes often differ and correlations becomes poorer with eluents containing high proportions of an organic modifier. A valuable aspect of this work has been the use of HPLC to readily determine $\log P$ values and other physicochemical parameters [5,7,8].

The second approach is to consider the retention as the summation of retention properties of the individual structural and functional groups in the analyte. This approach was first discussed by Martin [9], who suggested that a substituent changes the partition coefficient by a factor that depends only on the nature of the substituent and the two phases. Thus the retention of an analyte can be described by the summation of the effects of the individual constituents of the molecule, expressed at τ_i values (eqn. 1)

$$\log k'_S = \log k'_P + \sum_{i=1}^n \tau_i \quad (1)$$

where $\log k'_P$ is the retention of a parent compound and $\log k'_S$ is the retention of the derivative carrying n substituents. These substituent values are related to their effects on other equilibria and rates and are recognised as examples of a linear free-energy relationship (LFER). The early work in this field, which concentrated on gas–liquid chromatography (GLC) and thin-layer chromatography (TLC), and the theoretical background to the studies have been reviewed by Kaliszan [1,3].

This paper reviews studies of functional group contributions in HPLC, concentrating on more recent work, and examines the ways in which the

empirical values are dependent on the position of the group in the analyte molecule, interactions with other functional groups, the eluent composition, and stationary phase. The approach has particular interest because of the possibility of providing *de nova* prediction methods, as it should be possible to derive retention relationships directly from the structural features of an analyte.

2. FUNCTIONAL GROUP CONTRIBUTION VALUES

The functional group contribution (τ_X) to the retention of a compound can be determined from the difference in the retentions of two analytes, which differ by the presence and absence of the functional group X of interest. The initial studies in liquid chromatography were based on ΔR_M values in TLC [10] but most work is now carried out using HPLC because of its higher discrimination and better reproducibility (eqn. 2).

$$\tau_X = \log k'_{R-X} - \log k'_{R-H} = \log(k'_{R-X}/k'_{R-H}) \quad (2)$$

Early studies in HPLC were reported by Riley *et al.* [11], who established that the τ values were directly related to Hansch partition constants (π values) and that the solvophobic theory provided an general framework for rationalization of many of the observations.

Although the group contribution is usually expressed as τ_X (from eqn. 2) the same property has been expressed as T_i , with the same definition, or as α values defined as eqn. 3.

$$\alpha = k'_{R-X}/k'_{R-H} \quad (3)$$

such that $\log \alpha = \tau_X$. Some workers have calculated contributions as $\ln \alpha$ values and these have been converted to τ values where appropriate. In order to obtain a standardised functional group contribution, which is independent of the mobile phase composition, the τ values in mixed organic–water eluents are often extrapolated to give τ_w , τ_s , or τ_{water} values in 100% water.

The τ values are closely related to the substituent contributions ($I_{S,X}$) to the retention indices of analytes in HPLC proposed by Smith and Burr [12] and Morishita *et al.* [13]. These were determined from the difference in the

retention indices of analytes on the addition of a substituent, such that $I_{S,X} = 100 (\tau_X/\tau_{\text{CH}_2})$.

2.1. Measurement of τ values

Because the effect of a functional group can be influenced by the presence of other functional groups in the analyte the measured value of τ in polyfunctional compounds represents a cumulative value of the effect of the functional group itself and of its interaction with other groups. As a consequence reported values of τ can differ according to their environment and this problem will be considered later in the review.

2.1.1. Methylene increments

The simplest group relationship is the methylene selectivity, which is the difference in retention between members of a homologous series. Because the same change in retention properties should occur on the addition of a methylene group irrespective of the length of the alkyl chain there should be a linear relationship between carbon number and $\log k'$ and this has been reported for a number of different homologues [14,15]. Recently a detailed study of the effect of the mobile and stationary phases on the capacity factors of aliphatic and aromatic homologous series been carried out by Heron and Tchaplá [16].

From the Martin equation, the increment should be the same for different series of homologues. However, small but significant differences in the values have been observed by Colin *et al.* [14]. Similar results have also been noted by Smith and co-workers [17,18] on comparing the slopes of the relationships between $\log k'$ and carbon number for homologous alkan-2-ones, nitroalkanes, alkylbenzenes and alkyl aryl ketones. In methanol–water (30:70), the retention group increments could be calculated for the alkan-2-ones (mean values of $\tau = 0.39$), nitroalkanes ($\tau = 0.41$) and alkyl aryl ketones ($\tau = 0.43$) and in tetrahydrofuran (THF)–water (20:80) as $\tau = 0.37$, 0.38 and 0.39, respectively. However, because a different set of homologues was considered in each case, these differences may have a similar cause to changes observed by Tomlinson *et al.* [19], who noted that the

methylene group contributions in a number of aliphatic homologous series increased with the chain length up to C₅.

Studies by Smith and co-workers have examined the changes in capacity factors over a wide range of eluent compositions for homologous alkyl aryl ketones and alkylbenzenes [12,17,20]. Using this data the corresponding changes in the methylene group contributions have been calculated (Table 1). The mean values for the two series, ethylbenzene to *n*-butylbenzene and acetophenone to hexanophenone, were compared with the eluent composition (Figs. 1 and 2). The influence of the proportion of methanol on the methylene group contribution was linear but the changes with the proportions of both acetonitrile and THF were curved. By using the respective linear and quadratic correlations, the contributions (τ_w) in 100% water as the eluent could be determined for the alkylbenzenes; methanol, $\tau_w = 0.64$; acetonitrile, $\tau_w = 0.64$ and THF, $\tau_w = 0.68$; mean $\tau_w = 0.65$ and for the alkyl aryl ketones, methanol, $\tau_w = 0.60$; acetonitrile, $\tau_w = 0.60$ and $\tau_w = \text{THF}$, 0.70, mean $\tau_w = 0.63$. If a linear extrapolation had been used for the acetonitrile results very different τ_w values of 0.46 and 0.44 would have been obtained for the two series. The calculated value would change markedly if a different group of

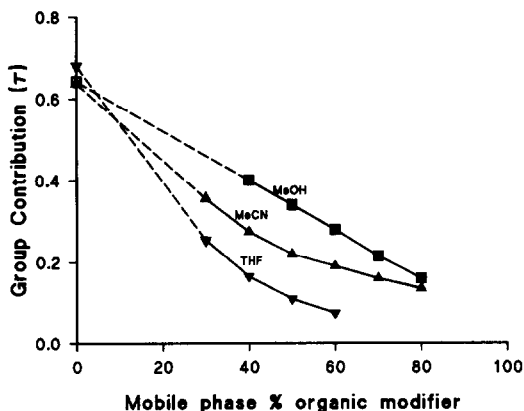


Fig. 1. Effect of proportion of organic modifier on the mean methylene group contributions from Table 1 for alkylbenzenes from ethylbenzene to *n*-butylbenzene. Extrapolation to give τ_w values for methanol with linear correlation and with acetonitrile and THF with quadratic extrapolation.

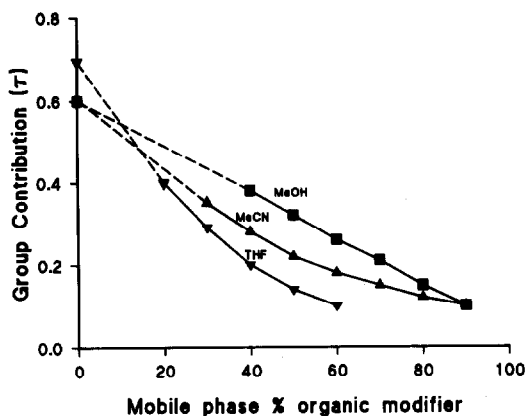


Fig. 2. Effect of proportion of organic modifier on the mean methylene group contributions from Table 1 for alkyl aryl ketones from acetophenone to hexanophenone. Extrapolation to give τ_w values for methanol with linear correlation and with acetonitrile and THF with quadratic extrapolation.

solvent composition values had been used in the correlation. Extrapolation of the correlation curves for the alkyl aryl ketones to the neat organic solvents gives 100% methanol, $\tau = 0.04$; 100% acetonitrile, $\tau = 0.09$ but THF, $\tau = 0.19$, which is greater than the value at THF–water (50:50). In the last case the extrapolation of a quadratic expression is too large for the value to be reliable.

The changes in retention from toluene to ethylbenzene was excluded from the calculations as they gave much smaller increments than for the higher homologues (Table 1). For example in methanol–water (50:50) the change ($\Delta \log k'$) for this increment was only 0.29 rather than 0.34 for the later homologues. This smaller increment was also reflected in smaller retention index increments for methylene substitution on a benzylic carbon (methanol and acetonitrile, 88 units and THF 86 units) compared to the defined value of 100 units for CH₂ [17,20] and in a non-linear increase in the values of the Hansch π contributions for alkyl substituents on a benzene ring (methyl, $\pi = 0.56$; ethyl, $\pi = 1.02$; propyl, $\pi = 1.55$; and butyl, 2.13) [21].

Gilpin *et al.* [22] have surveyed the methylene selectivities reported by a number of papers using eluents containing methanol, acetonitrile, or THF over a number of different composition

ranges. The analytes included *n*-alkanes, *n*-alkanols, *n*-alkanoates, *n*-alkylbenzenes and *n*-chloroalkanes. They extrapolated each data set to give the selectivity value [$s_w = \ln(k'_{n+1}/k'_n)$] in 100% water and found that almost all the values fell in the range 1.18–1.41 with a mean of 1.32 (which corresponds to τ_w values of 0.51–0.61 with a mean of 0.57) for CH_2 which are generally smaller than the values obtained above from the data of Smith and co-workers for alkylbenzenes and alkyl aryl ketones. Gilpin *et al.* also had to use quadratic extrapolations for the acetonitrile- and THF-containing eluents. They also calculated the methylene increment in neat organic modifiers but concluded that the values were very small ($\tau_{\text{organic}} < 0.1$ for methanol and THF and < 0.15 for acetonitrile) and because of the extrapolations might be subject to significant calculation errors.

In their study, Heron and Tchaplá [16] suggested that the differences in the retention between the modifiers can be explained by differences in the surface tensions of the eluents. These show a cross-over between methanol and acetonitrile at high modifier compositions and support the view that the retention is dominated by solvophobic effects.

In a recent study, Alvarez-Zepeda *et al.* [23] carried out a detailed thermodynamic study of methylene selectivity (reported as $\Delta \ln k'$) for ethylbenzene–hexylbenzene in methanol–water and acetonitrile–water eluents over the composition range 0% to 60% water. Typical values [expressed as $\tau = 0.35$ in methanol–water (50:50) and $\tau = 0.21$ in acetonitrile–water (50:50) at 25°C] corresponded closely to those derived from the study by Smith and Burr ($\tau = 0.34$ and $\tau = 0.22$, respectively at 30°C) [20]. Although there was a linear change in the selectivity ($\ln \alpha$ for CH_2) in methanol the relationship was curved in acetonitrile–water, however, the authors did not examine extrapolation of the curves to 100% water. In high proportions of the organic eluents the curves for methanol and acetonitrile intersected as in Figs. 1 and 2 and suggested that the τ values in neat eluent would be very small. By examining the changes with temperature they were able to measure the corresponding values of ΔH and ΔS .

2.1.2. Functional group increments

Surprisingly little work has been devoted to the determination of the group contribution of isolated functional groups either on an aromatic ring or on an aliphatic chain. Instead most studies have examined the effect of substituents on a skeleton which already contains polar functions, so that the functional group contribution implicitly also contains a component for electronic or steric interactions or for hydrogen bonding. This might be expected, as the driving force for much of the work on group contributions has been to compare the effects with QSAR studies of complex pharmaceutical compounds and related structures.

However, the capacity factors of many mono-substituted aromatic compounds have been reported in a variety of conditions and the functional group contributions can be readily calculated from the published results. The difficulty with using much of this data is that the conditions, in particular the temperature, have often not been carefully controlled and often only a single eluent or a limited range of compositions have been reported.

Two major sets of retention data have become available, which were each collected within a single laboratory and should therefore be internally consistent. In 1989 Smith initiated a project to express the group contributions of a range of substituents as retention index increments $I_{s,x}$, based on the alkyl aryl ketone scale [12]. Retention indices were chosen as these should be more robust than capacity factors to small changes in experimental conditions and column variations. The conditions were closely controlled and a single batch of stationary phase was used throughout the study at a controlled temperature, eluent pH and ionic strength [24]. The study by Smith and Burr initially examined single aromatic [12] and aliphatic substituents [20] in methanol and acetonitrile. This was extended to the examination of isomers [25] and disubstituted compounds [26] and led to the development of an expert system for retention index prediction (CRIPES) [27]. Subsequently the data were extended to separations using THF eluents by Smith and R. Wang [17] and more recently additional functional groups have been examined

by Smith and Y. Wang [28]. These studies were used above to calculate the methylene contributions.

Based on the data obtained by Smith and co-workers, the functional group contributions for a selection of substituents can be calculated for aromatic substituents in 40–80% methanol in water, 30–80% acetonitrile in water and 20–60% THF in water (Tables 2–4). The relative magnitudes of the contributions were generally similar to the corresponding Hansch substituent π contributions (Table 2) [21]. The τ values in all the tables are listed in the order of the decreasing Hansch π values so that any changes in selectivity can be readily noted. Significant changes in the values occur with the eluent composition, particularly for compounds which

give large polar or non-polar effects, and all the values tended to zero with increasing organic modifier. For eluent compositions, which give comparable capacity factors, there are also marked differences between the contribution of some groups. For example the τ values of cyano, nitro, acetyl and carbomethoxyl groups are -0.37 , -0.17 , -0.31 and -0.09 , respectively, in 70% methanol; -0.27 , -0.15 , -0.31 and -0.09 in 50% acetonitrile but -0.42 , -0.15 , -0.53 and -0.24 in 30% THF.

In an earlier study, Schoenmakers *et al.* [29] had collected capacity factors over a wide range of ternary eluent compositions to examine solvent selectivity, although the temperature and eluent pH were not specifically controlled. The corresponded functional group contributions can

TABLE 2
FUNCTIONAL GROUP CONTRIBUTIONS FOR AROMATIC SUBSTITUENTS MEASURED IN METHANOL-WATER

Based on capacity factors from Smith and co-workers [12,28]. Conditions as in Table 1.

Functional group	Group contribution (τ)					π^a
	Methanol (%)					
	40	50	60	70	80	
Ph	1.25	0.93	0.79	0.62	0.48	1.96
Br	0.56	0.43	0.33	0.27	0.19	0.86
CH ₂ Br	0.44	0.26	0.22	0.16	0.16	0.79
Cl	0.45	0.33	0.26	0.20	0.13	0.71
CH ₃	0.41	0.27	0.28	0.23	0.19	0.56
CH ₂ Cl	0.33	0.17	0.15	0.09	0.06	0.17
F	0.09	-0.03	0.01	-0.02	-0.02	0.14
H	0.00	0.00	0.00	0.00	0.00	0.00
CO ₂ CH ₃	0.09	-0.04	-0.09	-0.09	-0.10	-0.01
OCH ₃	0.03	-0.08	-0.03	-0.03	-0.01	-0.02
NO ₂	-0.15	-0.20	-0.19	-0.17	-0.16	-0.28
COCH ₃	-0.23	-0.36	-0.34	-0.31	-0.27	-0.55
CN	-0.36	-0.42	-0.42	-0.37	-0.33	-0.57
CH ₂ CN	-0.38	-0.51	-0.47	-0.46	-0.35	-0.57
CHO	-0.39	-0.48	-0.42	-0.37	-0.29	-0.65
OH	-0.70	-0.76	-0.66	-0.61	-0.50	-0.67
NHCOCH ₃	-0.74	-0.81	-0.68	-0.62	-0.40	-0.97
CH ₂ OH	-0.70	-0.75	-0.66	-0.55	-0.42	-1.03
NH ₂	-0.82	-0.83	-0.72	-0.61	-0.51	-1.23
CONH ₂	-0.99	-1.03	-0.93	-0.78	-0.64	-1.49
SO ₂ NH ₂	-1.21	-1.23	-1.06	-0.92	-0.58	-1.82

^a From ref. 21. Functional groups throughout paper arranged in descending order of Hansch π constants. For additional values used in other Tables see Table 23.

TABLE 3

FUNCTIONAL GROUP CONTRIBUTIONS FOR AROMATIC SUBSTITUENTS MEASURED IN ACETONITRILE-WATER

Based on capacity factors from Smith and co-workers [12,28]. Conditions as in Table 1.

Functional group	Group contribution (τ)					
	Acetonitrile (%)					
	30	40	50	60	70	80
Ph	1.09	0.85	0.64	0.50	0.39	0.32
Br	0.45	0.35	0.27	0.22	0.19	0.15
CH ₂ Br	0.44	0.30	0.22	0.14	0.12	0.11
Cl	0.36	0.27	0.21	0.17	0.14	0.11
CH ₃	0.39	0.26	0.26	0.17	0.17	0.15
CH ₂ Cl	0.34	0.23	0.16	0.09	0.07	0.03
F	0.10	0.01	0.05	-0.02	0.00	-0.02
H	0.00	0.00	0.00	0.00	0.00	0.00
CO ₂ CH ₃	-0.07	-0.10	-0.09	-0.10	-0.09	-0.08
OCH ₃	0.03	0.02	0.00	-0.01	-0.04	-0.03
NO ₂	-0.14	-0.15	-0.15	-0.15	-0.15	-0.15
COCH ₃	-0.38	-0.35	-0.31	-0.27	-0.23	-0.18
CN	-0.33	-0.30	-0.27	-0.25	-0.23	-0.20
CH ₂ CN	-0.26	-0.27	-0.25	-0.26	-0.23	-0.22
CHO	-0.37	-0.33	-0.28	-0.25	-0.22	-0.24
OH	-0.69	-0.65	-0.54	-0.51	-0.46	-0.40
NHCOCH ₃	-0.86	-0.87	-0.67	-0.62	-0.52	-0.45
CH ₂ OH	-0.85	-0.76	-0.63	-0.55	-0.47	-0.35
NH ₂	-0.75	-0.61	-0.53	-0.45	-0.39	-0.31
CONH ₂	-1.18	-1.03	-0.90	-0.77	-0.66	-0.42
SO ₂ NH ₂	-1.05	-1.02	-0.78	-0.73	-0.65	-0.66

again be calculated and when the results (Tables 5-7) are compared, there is a good comparison with those from Smith and Burr, even though they were obtained on different systems. The cyano, nitro and acetyl groups again showed marked relative changes from -0.44, -0.23 and -0.35, respectively, in 70% methanol to -0.25, -0.10 and -0.29 in 50% acetonitrile and -0.43, -0.16 and -0.59 in 30% THF. The largest differences between the two studies were for the more polar substituents and in many cases the differences may be caused by the uncertainties in the measurements and calculation of very small capacity factors (often less than 1).

No systematic work has been reported on the functional group contributions for groups substituted on saturated aliphatic chains, other than the addition of alkyl and hydroxyl groups. How-

ever, the τ values for a number of aliphatic groups (Table 8) can be calculated from the capacity factors for substituted alkylbenzenes reported by Smith and co-workers as part of their retention index study [20,28]. On comparing the substituents on toluene and ethylbenzene, the proximity to the phenyl group appeared to have only a small effect on the value of substituents. As with the calculation of the corresponding retention index increments [20] the contributions from the longer side chain are regarded as probably being the more typical of an isolated functional group and are used in subsequent calculations. There were differences between different eluent modifiers and the values generally decreased in magnitude with increased percentage of modifier. The τ values for the aliphatic substituents (R-X) differ markedly

TABLE 4

FUNCTIONAL GROUP CONTRIBUTIONS FOR AROMATIC SUBSTITUENTS MEASURED IN THF-WATER

Based on capacity factors from Smith and co-workers [17,28]. Conditions as in Table 1.

Functional group	Group contribution (τ)				
	THF (%)				
	20	30	40	50	60
Ph		0.65	0.38	0.21	0.11
Br	0.55	0.32	0.18	0.09	0.05
Cl	0.46	0.27	0.15	0.07	0.04
CH ₃	0.37	0.25	0.17	0.12	0.09
F	0.11	0.06	0.01	-0.01	-0.02
NHCH ₂ CH ₃	0.00	0.02	-0.00	-0.03	-0.05
H	0.00	0.00	0.00	0.00	0.00
CO ₂ CH ₃	-0.18	-0.24	-0.24	-0.21	-0.19
OCH ₃	-0.05	-0.09	-0.10	-0.10	-0.09
NO ₂	-0.13	-0.15	-0.17	-0.18	-0.18
COCH ₃	-0.58	-0.53	-0.44	-0.36	-0.30
OCOCH ₃		-0.39	-0.33	-0.27	-0.23
CN	-0.46	-0.42	-0.36	-0.30	-0.27
CHO	-0.60	-0.52	-0.42	-0.34	-0.28
OH	-0.49	-0.45	-0.40	-0.36	-0.34
NHCOCH ₃		-0.91	-0.80	-0.68	-0.62
NH ₂	-0.85	-0.69	-0.54	-0.43	-0.35
CONHCH ₃	-1.34	-1.22	-1.02	-0.86	-0.74
CONH ₂	-1.40	-1.26	-1.06	-0.84	-0.77
CON(CH ₃) ₂	-1.41	-1.27	-1.05	-0.86	-0.74
SO ₂ NH ₂	-1.16	-0.99	-0.81	-0.67	-0.59

from those calculated for the corresponding aryl substituents (Ar-X), for example in 40% methanol the τ values for Ar-OH and Ar-CN were -0.70 and -0.36, respectively, but for R-OH and R-CN were -1.29 and -1.04. They also changed more markedly with eluent composition and in 80% methanol the corresponding τ values for Ar-OH and Ar-CN were -0.50 and -0.33 but for R-OH and R-CN were -0.74 and -0.67.

The corresponding values for hydroxyl substitution on toluene and ethylbenzene can be calculated for the data of Schoenmakers *et al.* [29] and are found to be closely similar (Table 9).

Morishita *et al.* [13] carried out a closely related study based on retention indices derived from an *n*-alkane scale. They determined the retention index increments (δI_x) in methanol-

water (70:30) for individual functional groups (δI_x values for methyl, 89.3; phenolic hydroxyl, -195.7; arylamino, -213.6 and arylnitro, -61.9) and corrections for interactions of 2-, 3- and 4-substituted toluenes and nitroanilines. These increments were then used to predict the retention indices of a range of additional compounds. As part of a series of studies on the retention of aromatic hydrocarbons Popl *et al.* [30] have measured retention indices using a polynuclear aromatic hydrocarbon scale. From the results they calculated $\Delta \log I$ values for a wide range of substituents on the benzene ring using a polystyrene-divinylbenzene column. These differed for methanol-water and acetonitrile-water eluents. However, in both these retention index studies the capacity factors were not reported so that the corresponding τ values cannot be calculated, although the relative magnitudes of the

TABLE 5

FUNCTIONAL GROUP CONTRIBUTIONS FOR AROMATIC SUBSTITUENTS MEASURED IN METHANOL-WATER

Based on capacity factors from Schoenmakers *et al.* [29]. Conditions: column, Nucleosil 10 RP-18.

Functional group	Group contribution (τ)							
	Methanol (%)							
	30	40	50	60	70	80	90	100
OPh			0.80	0.58	0.41			
Ph			0.87	0.70	0.54	0.42	0.20	
(CH ₂) ₄				1.05	0.85	0.29	0.16	
COPh			0.51	0.34	0.19	0.08	0.04	-0.01
CH ₂ CH ₃			0.54	0.46	0.36	0.28	0.14	
Cl			0.37	0.30	0.24	0.15	0.12	0.05
CH ₃			0.36	0.29	0.25	0.21	0.16	0.09
N(CH ₃) ₂		0.29	0.22	0.15	0.12	0.09		
H	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CO ₂ CH ₃	0.12	0.02	-0.01	-0.06	-0.05			
OCH ₃		(0.45) ^a	0.03	-0.02	-0.03	-0.03	-0.03	-0.01
NO ₂	-0.14	-0.20	-0.20	-0.22	-0.23	-0.23	-0.19	-0.14
NHCH ₃		-0.23	-0.17	-0.21	-0.23	-0.22	-0.14	
COCH ₃	-0.20	-0.30	-0.28	-0.37	-0.35	-0.30	-0.27	-0.14
CN	-0.34	-0.43	-0.47	-0.46	-0.44	-0.43	-0.36	-0.23
CHO	-0.38	-0.44	-0.36	-0.46	-0.42	-0.36	-0.29	-0.18
OH	-0.67	-0.70	-0.71	-0.64	-0.62	-0.58	-0.47	-0.33
CH ₂ OH	-0.68	-0.70	-0.58	-0.67	-0.62	-0.52	-0.45	-0.30
NH ₂	-0.78	-0.71	-0.75	-0.64	-0.61	-0.54	-0.47	-0.30
(CH ₂) ₂ OH	-0.34	-0.42	-0.47	-0.47	-0.45	-0.41	-0.38	-0.26
(CH ₂) ₃ OH	0.02	-0.10	-0.13	-0.25	-0.27	-0.30	-0.29	-0.21

^a Value ignored.

contribution in the different systems were similar.

A number of other authors have reported sets of retention data measured at selected eluent compositions. Using these values the corresponding values of the group contributions can be calculated and compared with those obtained over wider eluent ranges. In a typical example, τ values (Table 10) can be calculated from capacity factors reported by Haky and Young [31] and compared with values derived from Tables 2 and 5. A close correspondence can be seen for the functional groups. The methylene increment between acetophenone and propiophenone ($\tau_{\text{CH}_2} = 0.31$) is similar to the value of $\tau = 0.28$, which can be estimated from Table 1 for methanol-buffer (55:45). Thus although there are

differences between the precise values of increments measured on different column systems (see later) the relative values are generally similar.

An important contribution to the ideas of functional group increments was the work of Jandera who suggested that the effect of changing the mobile phases could be divided into non-specific (n_{ce}) and specific or polar components (q_i) [32]. It was shown that Δq values are largely independent of the number and type of alkyl groups in the analyte but are related to the functional groups [33]. Subsequently functional group contributions for each factor, Δn_{ce} and Δq_i , were determined and used for retention prediction of phenylurea herbicides [34].

TABLE 6

FUNCTIONAL GROUP CONTRIBUTIONS FOR AROMATIC SUBSTITUENTS MEASURED IN ACETONITRILE-WATER

Based on capacity factors from Schoenmakers *et al.* [29]. Conditions: column, Nucleosil 10 RP-18.

Functional group	Group contribution (τ)						
	Acetonitrile (%)						
	30	40	50	60	70	80	90
OPh				0.75	0.83	0.53	0.31
Ph		0.76	0.61	0.48	0.42	0.34	0.26
(CH ₂) ₄	0.50	0.39	0.33	0.29	0.24	0.19	
COPh		0.38	0.28	0.21	0.18	0.13	0.09
CH ₂ CH ₃	0.50	0.40	0.34		0.39		
Cl	0.38	0.28	0.22	0.19	0.17	0.15	0.12
CH ₃	0.34	0.26	0.22	0.17	0.16	0.15	0.12
H	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CO ₂ CH ₃	-0.02	-0.07	-0.08	-0.07	-0.07	-0.06	-0.03
OCH ₃	0.00	-0.03	-0.02	-0.03	-0.04	-0.03	-0.03
NO ₂	-0.09	-0.09	-0.10	-0.11	-0.13	-0.13	-0.13
COCH ₃	-0.34	-0.33	-0.29	-0.27	-0.24	-0.20	-0.16
CN	-0.27	-0.27	-0.25	-0.23	-0.21	-0.22	-0.21
CHO	-0.39	-0.36	-0.32	-0.28	-0.24	-0.22	-0.18
OH	-0.71	-0.64	-0.60	-0.56	-0.53	-0.52	-0.48
CH ₂ OH	-0.85	-0.77	-0.72	-0.62	-0.55	-0.50	-0.40
(CH ₂) ₂ OH	-0.64	-0.62	-0.59	-0.55	-0.50	-0.38	-0.31
(CH ₂) ₃ OH	-0.36	-0.41	-0.42	-0.38	-0.36	-0.30	-0.19

2.2. Effect of interactions between functional groups and of the position of the substituent within the analyte molecule

2.2.1. Studies on complex molecules

Most analytes contain more than one functional group and these often interact electronically or by hydrogen bonding. In this situation the τ values which are derived from the difference between analytes with and without the functional group will represent a summed value representing the inherent group functional contribution and interaction contributions. The values can be related to chromatographic properties or biological activities but cannot be readily applied to other positions on the parent structures so have limited value in prediction. However, some studies have attempted to differentiate between

the two contributions and these are discussed in the next section. Multivariate analysis has been used to unravel the different contributions from the different groups when combination of groups are present in the analyte.

Typical studies of polyfunctional compounds have examined the effect of the introduction of a second substituent onto a benzene ring. Tomlinson *et al.* [19] examined the τ values for methoxyl, nitro and chloro groups on alkyl benzoates using different stationary phases and eluents. They also measured the thermodynamic parameters associated with changes in temperature and related the values to the compensation temperature. They reported noticeable differences in the τ values for 2-substituents compared to 3- and 4-substituents which were attributed to *ortho* interactions. In methanol-water (60:40) the contributions for the methoxyl groups were 2-, $\tau =$

TABLE 7

FUNCTIONAL GROUP CONTRIBUTIONS FOR AROMATIC SUBSTITUENTS MEASURED IN THF-WATER

Based on capacity factors from Schoenmakers *et al.* [29]. Conditions: column, Nucleosil 10 RP-18.

Functional group	Group contribution (τ)					
	THF (%)					
	30	40	50	60	70	80
COPh	0.14	0.01	-0.06	-0.12	-0.18	-0.23
Ph		0.38	0.25	0.14	0.06	0.00
(CH ₂) ₄	0.42	0.21	0.13	0.06	0.03	-0.02
Cl	0.29	0.14	0.09	0.04	0.03	0.02
CH ₂ CH ₃		0.35	0.26	0.19	0.15	0.12
CH ₃	0.27	0.18	0.15	0.11	0.09	0.10
N(CH ₃) ₂	0.16	0.20	0.12	0.13	0.08	0.00
H	0.00	0.00	0.00	0.00	0.00	0.00
OCH ₃	-0.11	-0.10	-0.11	-0.12	-0.11	-0.11
NO ₂	-0.16	-0.17	-0.19	-0.21	-0.22	-0.23
NHCH ₃	-0.09	0.01	-0.06	0.00	-0.08	-0.18
COCH ₃	-0.59	-0.48	-0.42	-0.41	-0.38	-0.35
CN	-0.43	-0.37	-0.36	-0.34	-0.36	-0.43
CHO	-0.57	-0.46	-0.39	-0.36	-0.34	-0.39
OH	-0.43	-0.40	-0.38	-0.41	-0.42	-0.43
CH ₂ OH	-0.86	-0.72	-0.65	-0.65	-0.70	-0.65
NH ₂	-0.44	-0.36	-0.37	-0.23	-0.28	-0.35
(CH ₂) ₂ OH	-0.66	-0.55	-0.51	-0.49	-0.47	-0.39
(CH ₂) ₃ OH	-0.47	-0.51	-0.51	-0.51	-0.59	-0.65

0.315; 3-, $\tau = 0.080$ and 4-, $\tau = 0.050$, for the nitro groups 2-, $\tau = 0.384$; 3-, $\tau = -0.075$ and 4-, -0.113 and for the chloro groups 2-, $\tau = 0.030$, 3-, $\tau = 0.461$ and 4-, $\tau = 0.405$.

In a similar study Butte *et al.* [35] determined $\log k^0$ values for a series of 3- and 4-substituted phenols by extrapolation of $\log k'$ values to 100% water from a range of methanol-water eluents. They used these values to calculate $\Delta \log k^0$ values, which represent τ values at 100% water, and showed that these could be related to Hansch π values. However, multiple substituted phenols gave poorer correlations and they omitted 2-substituted phenols because of the possibility of *ortho* interactions.

The effective values for a number of substituents on substituted aromatic rings over a wide range of eluent compositions can also be derived from the capacity factors measured by

Schoenmakers *et al.* [29]. The effective contribution of the second functional group was markedly dependent on the position and nature of the existing group (Table 11) (corresponding values could also be calculated for acetonitrile-water eluents). The contribution for a nitro group in methanol-water (50:50) changed from mildly hydrophilic when substituted on benzene ($\tau = -0.20$, from Table 5) to neutral when substituted on in the 2- or 3-position of nitrobenzene ($\tau = -0.02$ and -0.05 , respectively) to significantly hydrophobic when substituted on phenol ($\tau = 0.25$ to 0.51). If the strongly hydrophilic hydroxyl group is regarded as the substituent, it was found to have no effect in the 2-position on nitrobenzene and only a weak effect in the 3- and 4-positions. The first of these effects can be attributed to a degree of hydrogen bonding and the latter to electronic effects. The hydroxyl

TABLE 8

FUNCTIONAL GROUP CONTRIBUTIONS FOR ALIPHATIC SUBSTITUENTS

Based on capacity factors from Smith and co-workers [20,28]. Conditions as in Table 1.

Functional group	Group contribution (τ)										Fragmental constant (f) [21]						
	Methanol (%)					Acetonitrile (%)					THF (%)						
	40	50	60	70	80	90	30	40	50	60	70	80	90	30	40	50	60
$Ph(CH_2)_2X$																	
Br	0.03	-0.01	-0.05	-0.07	-0.10	-0.13	0.01	-0.03	-0.06	-0.09	-0.10	-0.11	-0.04	-0.05	-0.06	-0.06	-0.08
Cl	-0.08	-0.10	-0.13	-0.13	-0.16	-0.15	-0.10	-0.13	-0.14	-0.15	-0.16	-0.16	-0.12	-0.09	-0.12	-0.14	-0.14
CN	-0.79	-0.78	-0.75	-0.68	-0.54	-0.52	-0.65	-0.53	-0.51	-0.43	-0.39	-0.29	-0.37	-0.39	-0.37	-0.21	-0.21
OH	-1.11	-1.02	-0.94	-0.78	-0.61	-0.55	-1.24	-1.02	-0.90	-0.72	-0.64	-0.50	-0.50	-0.64	-0.50	-0.26	-0.26
$Ph(CH_2)_2X$																	
Br	-0.02	-0.04	-0.05	-0.10	-0.10	-0.13	0.01	-0.03	-0.06	-0.09	-0.10	-0.11	-0.04	-0.05	-0.06	-0.06	-0.08
Cl	-0.15	-0.16	-0.19	-0.16	-0.15	-0.15	-0.10	-0.13	-0.14	-0.15	-0.16	-0.16	-0.12	-0.16	-0.12	-0.14	-0.14
CO_2CH_3	-0.43	-0.46	-0.44	-0.40	-0.35	-0.35	-0.47	-0.43	-0.39	-0.36	-0.33	-0.29	-0.37	-0.39	-0.37	-0.21	-0.21
$COCH_3$	-0.71	-0.72	-0.72	-0.57	-0.50	-0.50	-0.66	-0.65	-0.48	-0.45	-0.42	-0.44	-0.44	-0.42	-0.44	-0.26	-0.26
CN	-1.01	-0.96	-0.91	-0.78	-0.66	-0.66	-0.80	-0.71	-0.53	-0.56	-0.51	-0.46	-0.50	-0.42	-0.44	-0.26	-0.26
OCH_3	-0.55	-0.55	-0.52	-0.40	-0.34	-0.34	-0.56	-0.55	-0.40	-0.33	-0.29	-0.30	-0.37	-0.39	-0.37	-0.21	-0.21
OH	-1.20	-1.05	-1.00	-0.83	-0.67	-0.67	-1.34	-1.16	-0.98	-0.84	-0.70	-0.57	-0.57	-0.70	-0.57	-0.26	-0.26
$CONH_2$	-1.39	-1.30	-1.19	-0.99	-0.81	-0.81	-1.57	-1.40	-1.20	-1.03	-0.87	-0.69	-0.69	-0.87	-0.79	-0.64	-0.64
$Ph(CH_2)_3X$																	
Br																	
Cl																	
CN																	
OH																	

TABLE 9

FUNCTIONAL GROUP CONTRIBUTIONS FOR ALIPHATIC SUBSTITUENTS

Based on capacity factors from Schoenmakers *et al.* [29]. Conditions as in Table 5. 1-OH = Benzyl group; 2-OH = substituent on ethylbenzene.

Functional group	Group contribution (τ)										Fragmental constant (f) [21]							
	Methanol (%)					Acetonitrile (%)					THF (%)							
	50	60	70	80	90	30	40	50	60	70	80	90	30	40	50	60	70	80
1-OH	-0.93	-0.95	-0.87	-0.72	-0.62	-0.62	-1.19	-1.03	-0.93	-0.78	-0.71	-0.65	-0.52	-1.12	-0.90	-0.79	-0.75	-0.79
2-OH	-1.01	-0.91	-0.91	-0.77	-0.66	-0.66	-1.12	-1.00	-0.88	-0.88	-0.70	-0.70	-0.70	-0.90	-0.77	-0.68	-0.62	-0.51

TABLE 10
FUNCTIONAL GROUP CONTRIBUTIONS FOR SUBSTITUENTS ON AN AROMATIC RING

Based on data from Haky and Young [31]. Conditions: column, Alltech RP-18; eluent, methanol-pH 7.0 buffer (55:45).

Functional group	Group contribution (τ)	$\log P$ (PhX)
OPh	0.903	4.21
Ph	0.943	4.06
C ₃ H ₇	0.953	3.62
(CH ₂) ₄	0.603	3.37
OC ₃ H ₇	0.613	3.18
COPh	0.393	3.18
Br	0.393	2.99
Cl	0.313	2.49
CH ₃	0.333	2.74
CH ₂	0.310	
CO ₂ C ₂ H ₅	0.243	2.64
OC ₂ H ₅	0.233	2.51
OCH ₃	-0.024	2.08
COC ₂ H ₅	-0.076	2.20
H	0.000	2.01
CO ₂ CH ₃	-0.037	2.18
NHC ₃ H ₇	0.233	2.45
CH ₂ OCOCH ₃	-0.077	1.96
CH=CHCN	-0.207	1.96
CH=CHOH	-0.298	1.95
OCOCH ₃	-0.347	1.49
NHCH ₃	-0.350	1.66
COCH ₃	-0.381	1.66
CN	-0.466	1.56
CH ₂ CN	-0.504	1.56
CH ₂ OH	-0.676	1.16
NHCOCH ₃	-0.723	1.16
CSNH ₂	-0.754	1.49
NHCHO	-0.767	1.12
NH ₂	-0.805	0.90
CONH ₂	-1.088	0.65

group showed a more marked effect [$\tau = -0.54$ in methanol-water (50:50)] in the 4-position of chlorobenzene. The fluoro group, whose effect on benzene was neutral (Table 2), had a larger hydrophobic effect in the 3-position of nitrobenzene than in the 2- or 4-positions. The introduction of a second carbomethoxyl group into the 2-position of methyl benzoate to give dimethyl phthalate had a surprisingly hydrophilic effect [$\tau = -0.28$ in methanol-water (50:50)] compared with $\tau = -0.01$ for a carbomethoxyl

group on benzene in Table 5). This is probably caused partly by a steric effect reducing conjugation of the carbonyl groups with the aromatic ring.

Significant differences in the effect of a substituent caused by the position of substitution on phenols was also noted by Smith and Burr [26] in their studies using retention index increments (e.g. for a chloro group on benzene, the substituent index (I_s) = 105 but 2-Cl on phenol, $I_s = 99$; 3-Cl, $I_s = 149$ and 4-Cl, $I_s = 138$). Very marked changes occurred when the groups could undergo hydrogen bonding, for example the retention index increment of a carbomethoxyl group in methanol-water (50:50) changed from $I_s = -10$ for substitution on benzene to $I_s = 275$ for substitution in the 2-position of phenol, $I_s = 54$ in the 3-position and $I_s = 49$ in 4-position.

Riley *et al.* [11] measured the functional group contributions, using ion-pair chromatography, for substituents on phenyl rings carrying triazine or azapurine groups and on benzoic acids. In each case there was a good correlation between the τ values for the 3- and 4-substituents and Hansch π constants. The 3- and 4-substituents on the benzoic acids gave very similar values (for example: 3-NH₂, $\tau = -0.50$ and 4-NH₂, $\tau = -0.55$; 3-OH, $\tau = -0.30$ and 4-OH, $\tau = -0.45$; and 3-NO₂, $\tau = 0.16$ and 4-NO₂, $\tau = 0.15$) but were markedly different from the 2-substituents (2-NH₂, $\tau = -0.10$; 2-OH, $\tau = 0.25$; 2-NO₂, $\tau = -0.14$), which was attributed to *ortho* interactions with the carboxylic acid group.

More complex systems have also been examined, usually based on natural products, biologically active compounds or metabolites, in which substitution has taken place either on a ring or on an aliphatic side chain. From the examination of a wide range of protonated catecholamines, which were separated on an ODS column with pH 2.1 phosphate buffer, Molnár and Horváth [36] derived τ values for the 3-OH group (-0.53 or -0.24 with 4-H and 4-OH, respectively), 4-OH group (0.60 or -0.80, if no ionised amino group was present), 3-methoxyl (0.52) and α -OH group (-0.94 or -0.58 depending on the absence or presence of an ionised amino group), α -NH₃⁺ group (-0.89), β -NH₂⁺-CH₃ (0.29) and β -CH₂ (0.29). Although

TABLE 11

FUNCTIONAL GROUP CONSTANTS FOR SUBSTITUENTS ON SUBSTITUTED AROMATIC RINGS

Based on capacity factors from Schoenmakers *et al.* [29]. Conditions as in Table 5.

Functional groups		Group contribution (τ) for group Y on PhX				
X	Y	Methanol (%)				
		40	50	60	70	80
Cl	4-CH ₃			0.29	0.39	0.19
Cl	2-Cl			0.28	0.19	0.08
Cl	4-CHO		-0.96	-0.70	-0.04	-0.05
Cl	4-OH		-0.54	-0.50	-0.48	-0.38
CO ₂ CH ₃	2-CO ₂ CH ₃	-0.17	-0.28	-0.26	-0.31	
NO ₂	2-NO ₂	0.00	-0.05	-0.04	-0.07	-0.10
NO ₂	3-NO ₂	0.00	-0.02	-0.03	-0.05	-0.05
NO ₂	4-NO ₂	-0.09	-0.13	-0.09	-0.06	-0.06
NO ₂	2-F	0.06	0.02	0.05	0.04	0.03
NO ₂	3-F	0.18	0.15	0.20	0.17	0.14
NO ₂	4-F	0.07	0.02	0.05	-0.05	0.09
NO ₂	2-OH	0.03	0.00	0.01	0.05	0.07
NO ₂	3-OH	-0.16	-0.19	-0.20	-0.15	-0.15
NO ₂	4-OH	-0.22	-0.26	-0.24	-0.21	-0.18
NO ₂	4-COCH ₃	-0.03	-0.08	-0.05	-0.04	-0.05
OH	4-Cl		0.53	0.51	0.40	0.39
OH	4-F	0.18	0.17	0.11	0.11	0.05
OH	2-NO ₂	0.53	0.51	0.45	0.44	0.42
OH	3-NO ₂	0.34	0.32	0.24	0.24	0.20
OH	4-NO ₂	0.29	0.25	0.20	0.18	0.17

some of these differences are due to the interaction of adjacent groups, the presence of a charged group elsewhere in the analyte has a marked effect. This study was subsequently extended by Chen and Horváth and the differences between three different ODS-silica columns was determined [37]. The related phenylethylamines (Ph- β CH₂- α CH₂-NR₂) were studied by Gill *et al.* [38] using a methanol-pH 3.15 buffer (10:90), containing diethylamine as a masking agent. They found that the addition of methyl had a larger effect in the α -position ($\tau = 0.36$ – 0.44) than on nitrogen ($\tau = 0.02$ – 0.13). Hydroxyl substitution caused a reduction in retention (β -OH, $\tau = -0.25$ to -0.35) and *p*-Ph-OH, $\tau = -0.38$ to -0.89). The smaller values for the β -substituent probably reflected hydrogen-bonding to the terminal amino group.

The effects of the position of substitution were

also observed by Huang *et al.* [39], who examined a series of methoxylated benzoquinones and *p*-hydroquinones. They used the 2-methoxyl derivatives as the parent compounds and determined the effects of substitution in the 3-, 5- and 6-positions with further methoxyl groups. They concluded that simple additive effects were not sufficient to model the system and that a factor to represent *ortho* interactions would be needed.

Other studies have examined substituted heterocyclic systems in which there can be interaction both with other substituents and the hetero atom. Assenza and Brown [40] examined 86 substituted purines and measured T_i values (defined as $\ln k'_i - \ln k'_p$) from separations carried out using 100% pH 5.7 buffer. They noted that in many cases there may be interactions with other parts of the analytes and thus the T_i values

TABLE 12
FUNCTIONAL GROUP CONTRIBUTIONS FOR SUBSTITUENTS ON A PYRIDINE RING

Based on data from Gago *et al.* [41]. Conditions: column, Supelco LC18; eluent, methanol-0.015 M triethylamine (45:55).

Functional group	Group contribution (τ)		
	Position		
	2-	3-	4-
Phenyl			1.15
<i>tert.</i> -Butyl			1.18
Br	0.32	0.51	0.51
Cl	0.23	0.41	0.42
CH ₃	0.23	0.32	0.32
F	0.01	0.09	
H	0.00	0.00	0.00
CO ₂ CH ₃	-0.38	0.12	0.17
OCH ₃	0.39		0.18
COCH ₃	0.09	-0.20	-0.15
CN	-0.21	-0.27	-0.24
CHO	-0.21	-0.37	-0.41
NH ₂	-0.20	-0.54	-0.15
CONH ₂	-0.39	-0.89	-0.89

may also include a component for these interactions. For example the T_j value for a methyl group ranged from 0.82 to 1.70 (equivalent to τ values = 0.36 to 0.74) depending on its location. The values determined on two C₁₈ columns and a C₈ column showed large differences but were co-linear. However, they did not correlate well with a trimethylsilyl-silica column.

Gago *et al.* [41] have reported $\log k'$ for a wide range of monosubstituted pyridines, which they used to determine π values by comparison with empirical $\log P$ measurements. Using their capacity factor measurements the corresponding functional group contributions have been calculated (Table 12). In many cases the 2-, 3- and 4-substituents gave similar τ values, and these were closely related to the values for aromatic substituents (Table 2). For substituents containing a carbonyl group (formyl, acetyl and amido) the 2-substituent had a less negative value than the 3- and 4-substituents. In contrast, the 2-carbomethoxyl group had a much more negative effect and this was reflected in a much lower $\log P$ value (3-CO₂CH₃, $\log P = 0.81$ and 2-CO₂CH₃, $\log P = 0.27$). The 3-amino group was

TABLE 13
FUNCTIONAL GROUP CONTRIBUTION FACTORS FOR SUBSTITUTION ON FURAN

Based on capacity factors in Yamagami and Takao [42]. Conditions: Column, Capcell Pak C₁₈; eluent, methanol-pH 7.4 buffer.

Functional group	Group contribution (τ)						Log k_w
	Methanol (%)						
	0	5	15	30	50	70	
H	0.00	0.00	0.00	0.00	0.00	0.00	1.23
2-CH ₃	0.59	0.58	0.54	0.46	0.36	0.27	1.83
2-CH ₂ CH ₃		1.17	1.09	0.93	0.71	0.51	2.46
2-OCH ₃	0.49	0.39	0.30	0.19	0.08	0.01	1.55
2-COCH ₃	0.33	0.09	-0.18	-0.40	-0.53	-0.53	0.90
2-CO ₂ CH ₃	0.72	0.48	0.21	-0.02	-0.20	-0.28	1.38
3-CO ₂ CH ₃	0.80	0.58	0.38	0.16	-0.03	-0.13	1.59
2-CO ₂ CH ₂ CH ₃	1.29	1.00	0.70	0.39	0.10	-0.05	1.93
3-CO ₂ CH ₂ CH ₃	1.38	1.15	0.88	0.58	0.28	0.09	2.16
2-CON(CH ₃) ₂	0.69	0.35	-0.05	-0.37	-0.57	-0.60	1.00
3-CON(CH ₃) ₂	0.53	0.22	-0.16	-0.46	-0.64	-0.67	0.90
2-CONH ₂	-0.37	-0.57	-0.82	-0.99	-1.10	-1.16	0.35
3-CONH ₂	-0.43	-0.60	-0.80	-0.93	-1.01	-1.06	0.39

also very different from the 2- and 4-amino groups.

In an interesting study Yamagami and Takao [42] examined the effect of substitution in different positions on the furan ring and the corresponding functional group contributions can be calculated (Table 13). There were significant differences between the effect of substitution in the 2- and 3-positions and the values of the groups were often different from those obtained on an aromatic ring. As part of the same study they also examined the effect of the carbomethoxyl group on both oxygen and nitrogen heterocyclic rings (Table 14). Although in most cases the influence was small [usually τ between 0.20 and 0.10 in methanol-pH 7.4 buffer (30:70)] similar to substitution on benzene ($\tau = 0.17$), large hydrophobic changes in retention were observed for substitution in the 5-position of the pyrimidine ring ($\tau = 0.47$), substitution in the 4-position of the pyridazine ring ($\tau = 0.59$), and for substitution on the 2- and 3-positions of indole ($\tau = 0.64$ and 0.38).

Comparison of natural products and related compounds have been fruitful as often large families of closely related compounds are avail-

able with a common backbone. The influence of the position of a substituent on its contribution can be seen in the work of Dondi and co-workers [43,44], who have examined the effects of the substitutions of hydroxyl and glucosyl groups onto flavanoid structures. They found that $\Delta \log k'$ was largely independent of the stationary phase or of the acid used to alter the pH of the eluent. However, the τ value for a hydroxyl group differed markedly depending on its position (e.g. 3-OH, $\tau = -0.15$; 4-OH, $\tau = -0.97$; 6'-OH, $\tau = -0.97$; 2'-OH, $\tau = 0.40$; 3'-OH, $\tau = -0.24$; 4'-OH, $\tau = 0.34$). They felt that these differences could often be accounted for by the presence of interactions with other groups within the pairs of model compounds used for the determinations. For example, the values for the 3'-OH group were obtained from two compounds both containing a 4'-OH group and the 3-OH group may have a less negative effect than the 6-OH group because it can hydrogen-bond to the 4-carbonyl group. All the compounds studied also contained a 5-OH group which can also be involved in hydrogen-bonding. The work was continued in a more detailed study [45] and they found that "the effect were not strictly constant and additive but are a complex results of intramolecular interactions". They also examined correlations between the τ values and chemical shifts measured using ^{13}C nuclear magnetic resonance spectroscopy.

The $\Delta \log k'$ for substituents on coumarins have been determined for methanol-water (60:40) by Glowniak and Bieganowska [46] and were compared with ΔR_M values obtained by TLC. Walczak *et al.* [47] examined a series of chalcones over a range of eluents and mobile phases. They found that the separation factor $\alpha_{X-Y/X-H}$ (which is closely related to the group contribution) varied with the stationary phases and the position of substitution.

As well as electronic interactions, the steric position of substituents can affect their functional group contribution. In a study of sterols DiBussolo and Nes [48] found that the effect of hydroxyl substituents, expressed as σ values (k'_{R-X}/k'_{R-H}) were very dependent on their steric configuration (3 α -OH, $\sigma = 0.38$ equivalent to $\tau = -0.42$ and 3 β -OH, $\sigma = 0.53$ equivalent to $\tau = -0.27$). The effect was more marked if

TABLE 14

FUNCTIONAL GROUP CONTRIBUTIONS FOR SUBSTITUTION OF CARBOMETHOXYL GROUPS ON HETEROCYCLIC RINGS

Based on capacity factors in Yamagami and Takao [42]. Conditions: column, Capcell Pak C₁₈; eluent, methanol-pH 7.4 buffer.

	Functional group	Group contribution (τ)		
		Methanol (%)		
		30	50	70
Benzene	1-CO ₂ CH ₃	0.17	-0.04	-0.13
Pyrazine	2-CO ₂ CH ₃	0.20	0.09	-0.02
Pyrimidine	2-CO ₂ CH ₃	-0.07	-0.21	-0.31
	5-CO ₂ CH ₃	0.47	0.35	0.24
Pyridazine	4-CO ₂ CH ₃	0.59	0.45	0.34
Furan	2-CO ₂ CH ₃	-0.02	-0.21	-0.26
	3-CO ₂ CH ₃	0.17	-0.03	-0.13
Benzofuran	2-CO ₂ CH ₃	0.10	-0.07	-0.11
	2-CO ₂ CH ₃	0.64	0.38	0.22
Indole	2-CO ₂ CH ₃	0.64	0.38	0.22
	3-CO ₂ CH ₃	0.38	0.12	0.00

methanol was included in the eluent. Little effect was observed for methyl or alkyl groups in different positions or for changes caused by the introduction of double bonds.

The value of functional group substituents for

retention prediction of complex molecules has been examined by Valko *et al.* [49], who included 100 $\Delta \log k'$ values in a database for the retention prediction of metabolites. They ignored the effects of different eluents or changes with

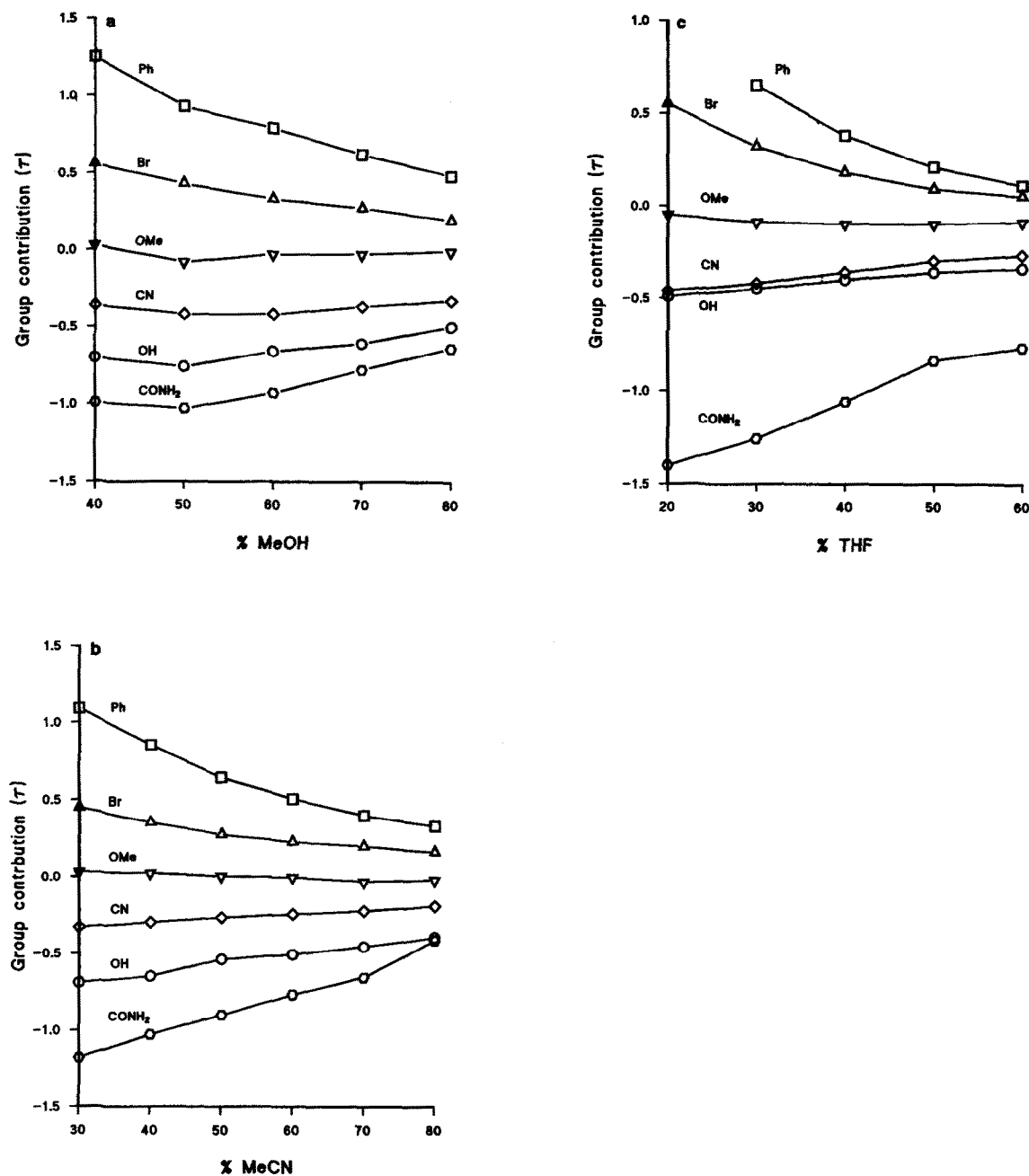


Fig. 3. Effect of proportion of modifier in the eluent on functional group contributions. Data from Tables 2-4. Modifier: (a) methanol; (b) acetonitrile; (c) THF.

composition and used average values calculated for C-hydroxylation, N-demethylation and decarboxylation (τ values: OH, -0.313 ; loss of NH-CH_3 , -0.162 and Ph-H to Ph-OH, -0.622) [49]. Although they excluded the effects of adjacent groups in their calculations, they acknowledged that interactions would occur and thus would cause an uncertainty in the results.

2.2.2. Studies of interaction components of τ values

In the studies of di- and polyunsaturated benzenes, Tsantili-Kakoulidou *et al.* [50] studied the differences T^* between the experimental $\log k'_w$ values and those calculated by the addition of substituent $\log k'_w$ values. The differences for *meta*- and *para*-substituents were related to the substituent interaction parameters ρ and σ suggested by Brändstrom [51] and Fujita [52] (eqn. 4).

$$T_I = \rho_X \sigma_Y + \rho_Y \sigma_X \quad (4)$$

They also examined *ortho*-substitution and considered additional parameters to cover steric interactions and weak or strong hydrogen-bonding effects.

In a closely related study Smith and Burr examined the interactions in a series of substituted phenols and toluenes [26]. They had to exclude a number of the compounds as they appeared to be ionised. Significant interactions between groups (expressed as retention index increments) were observed in many cases, particularly for *ortho*-substituents, and these were examined using a similar expression to eqn. 4. The magnitude of the interactions followed similar trends and the potential of using ρ and σ values as the basis of a general scheme for the prediction of interaction terms was explored. The work has subsequently been extended to eluents containing THF but the results suggest that the correlations are poorer probably because the relative magnitudes of the σ values had changed with the selectivity of the modifier [28].

Spanjer and De Ligny examined interactions in monosubstituted benzenes, chlorobenzenes, benzoic acids, phenols, anilines and pyridines

using methanol–water (50:50) and acetonitrile–water (30:70) eluents [53] and subsequently also with THF–water (32.5:67.5) as eluent [54]. The substituted benzenes gave a series of primary substituent constants ($\Delta \log V_N$ values, which are equivalent to τ values), which were then used to determine the secondary $\Delta \Delta \log V_N$ values due to interactions between substituents. These could be correlated with Hammett and Taft type functions.

Thus one problem with the use of group contributions as a method for predicting retention and in understanding retention is that the effective values in any situation can be dependent on the presence of *ortho* interactions, and hydrogen-bonding interactions with adjacent groups, and of longer range interaction across aromatic rings, although these can be modelled. However, some substitutions can also cause a change in the $\text{p}K_a$ of the product and the possibility of ionisation.

2.3. Effect of eluent composition on τ values

The magnitude of τ values usually depend on the nature and percentage of the organic modifier in the eluent and it is these changes, which provide the selectivity differences between modifiers and enable increased resolution to be obtained by solvent optimisation.

In early work, the functional group contributions were found to be linearly dependent on the eluent composition expressed as the surface tension [16,19]. However, when the τ values determined in Tables 2–4 are plotted against % composition (Fig. 3a–c) the relationships are often curved and an extrapolated value of τ_w would be dependent on the range of compositions selected for the calculation. The magnitude of the τ values decreased with an increase in the proportion of organic component in the mobile phase. The aliphatic substituents (Table 8) show similar changes with composition (Fig. 4).

When the whole range of substituents are compared in eluent compositions selected to give similar capacity factors for benzene [$k' = 7.37$ in methanol–buffer (50:50); $k' = 6.57$ in acetonitrile–water (40:60) and $k' = 5.24$ in THF–water (40:60)] the markedly different groups can be

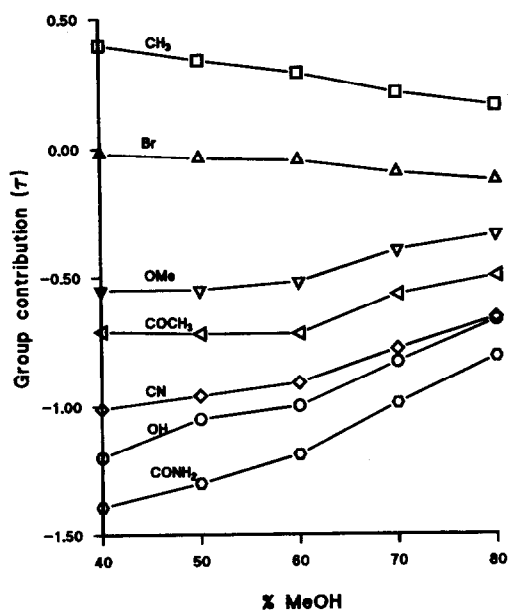


Fig. 4. Effect of proportion of modifier in the eluent on aliphatic functional group contributions. Data from Table 8. Modifier: methanol.

readily identified (Fig. 5a and b). The phenyl, halo and carbomethoxyl groups have significantly smaller increments in THF and the hydroxyl, amino and sulphonamide groups much large increments in THF compared to methanol. In contrast the group contributions for the polar carboxamide and N-acetyl groups were similar in both eluents. The difference between methanol and acetonitrile were not as marked but the sulphonamide amino and benzylcyano groups were the most different.

Similar differences can also be seen in other studies. Tanaka *et al.* [55] examined the k'_{R-X}/k'_{R-H} ratio for a number of monosubstituted aromatic compounds in methanol-water (50:50), acetonitrile-water (30:70) and THF-water (25:75), which had been selected because they gave roughly equal methylene increments. From their data the corresponding τ values can be calculated for a number of substituents (Table 15) and these show marked differences between the three eluents. This paper demonstrated the power of changing the organic modifier as the order of elution of a mixture of *p*-nitrophenol, *p*-dinitrobenzene, nitrobenzene and methyl ben-

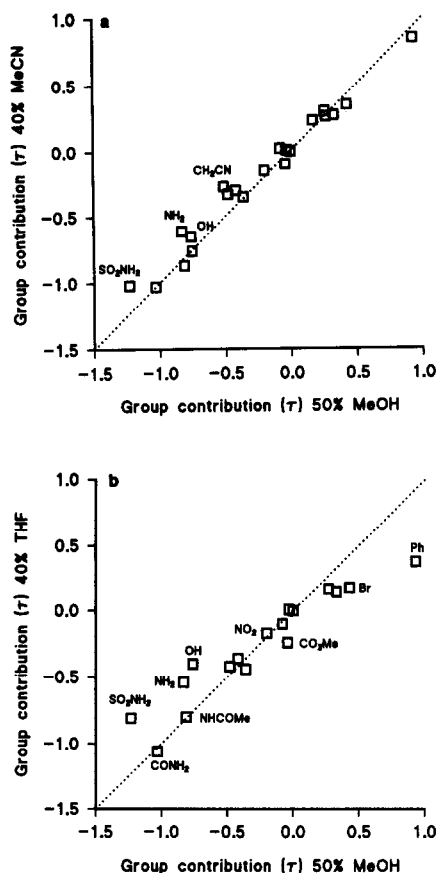


Fig. 5. Correlation of functional group contributions for aromatic substituents in isoelectrostatic eluents. Data from Tables 2-4. Eluent: (a) methanol-pH 7.0 buffer (50:50) compared to acetonitrile-pH 7.0 buffer (40:60); (b) methanol-pH 7.0 buffer (50:50) compared to THF-pH 7.0 buffer (40:60).

zoate was completely reversed on changing from methanol-water (50:50) to THF-water (25:75). The corresponding τ values for the nitro group were -0.19 and -0.10 , respectively, and for the carbomethoxyl group 0.01 and -0.19 .

In their study of methoxylated quinones and hydroquinones, Huang *et al.* [39] found that the empirical group contributions differed according to the organic modifier. For example, substitution of methoxyl in the 5 position gave $\tau = 0.526$ in methanol-water (20:80); $\tau = 0.179$ in acetonitrile-water (20:80) and $\tau = 0.956$ in THF-water (10:90), whereas for the 6-methoxyl substituent the corresponding values were $\tau = 0.390$, 0.136 and -0.047 , respectively.

TABLE 15
FUNCTIONAL GROUP CONTRIBUTIONS FOR SUBSTITUENTS ON BENZENE IN DIFFERENT ELUENTS CHOSEN TO GIVE A CONSTANT METHYLENE INCREMENT

Based on capacity factors from Tanaka *et al.* [55]. Column: endcapped octyl-bonded phase.

Functional group	Group contribution (τ)		
	Eluent		
	MeOH-water (50:50)	MeCN-water (30:70)	THF-water (25:75)
Cl	0.31	0.33	0.35
CH ₃	0.30	0.30	0.29
H	0.00	0.00	0.00
CO ₂ CH ₃	0.01	-0.04	-0.19
OCH ₃	-0.02	-0.01	-0.06
NO ₂	-0.19	-0.10	-0.10
COCH ₃	-0.30	-0.34	-0.51
CN	-0.33	-0.28	-0.38
CHO	-0.39	-0.39	-0.51
OH	-0.52	-0.64	-0.35
CH ₂ OH	-0.52	-0.80	-0.77
CONH ₂	-0.95	-1.15	-1.22

Dondi *et al.* [44] also reported considerable differences between measurements in eluents containing methanol, acetonitrile or THF for glycosyl and hydroxyl groups substituted on flavanoids. The increments (reported as $\Delta \log k'$) in methanolic solvents were relatively insensitive to the eluent composition but there was a marked dependence with THF and acetonitrile eluents. In both cases the values tended towards zero as the proportion of modifier increased. Over the limited eluent ranges that they studied, they reported significant differences in the mean $\Delta \log k'_w$ values for a number of groups in different modifiers, although the values were usually constant over a range of compositions, typically 30 or 40 to 55%. These groups included the 3-glycosyl group whose contribution changed from -0.44 in methanol to -0.78 in acetonitrile and -0.75 in THF, the 3-OH from -0.13 in methanol to 0.03 in acetonitrile and 0.13 in THF and the 3'-OH from -0.19 in methanol to -0.22 in acetonitrile and -0.07 in THF. The extrapolated $\Delta \log k'_w$ values calculated from the three

modifiers were found to be very similar. Because of the differences in the effects in the presence of the modifiers and the changes in the τ values with the proportion of acetonitrile and THF, they suggested that changing the mobile phase composition could provide a valuable method for improving the separation of these compounds.

As part of a study of cross-ring effects on τ values, Wells and Clark [56] measured the contributions for methyl, chloride, nitro and methoxyl groups for 4,4'-disubstituted benzamides and benzanilides on three different ODS-silica phases. As with Fig. 3, the τ values decreased with increasing proportions of acetonitrile in the mobile phase. They converted the empirical τ values into standardised τ_s values (at 100% water) to eliminate the effect of the mobile phase composition and compared these values with substituent constants derived from partition coefficients. The main differences between the analytes were found for the 4-nitro group, whose τ values ranged from 0.00 to 0.25 in acetonitrile-water (45:55). However, for the three analytes with a nitro group *para* to the amide carbonyl group, very similar τ values were found in each case. Although the τ_s values for the methyl group were virtually the same in each series of analytes ($\tau_s = 0.126$ to 0.130), there were small systematic differences for the methoxyl ($\tau_s = 0.005$ to 0.067) and chloro groups ($\tau_s = 0.146$ to 0.218). Larger differences between the analytes were found for the nitro group ($\tau_s = 0.036$ to 0.210).

This application of τ_w or τ_s values (representing an extrapolation to 100% water) to eliminate the effects of the organic modifier have been used in a number of studies particularly when comparisons are being made with other parameters. However, these parameters remove the differences induced by the modifier which are important in selectivity comparisons. The equivalence of extrapolated values obtained from different eluent modifiers can be demonstrated by examining the recent work of Altomare *et al.* [57], who measured $\log k'_w$ by linear regression for a series of 3- and 4-substituted benzenesulphonamides. Using these results they calculated the functional group contributions for the substituents in both methanol-water ($\tau_{w(M)}$)

and acetonitrile-water ($\tau_{w(A)}$) (Table 16). The values differed from the two modifiers but each set correlated closely to π values measured in an earlier study. No significant differences were found between the 3- and 4-substituents although the values often differed markedly from those determined below (Table 17) for substitution on benzene.

To test the extrapolation methods, the group contributions for substituents on benzene, calculated earlier for methanol, acetonitrile and THF from the work of Smith and co-workers (Tables 2-4), were linearly extrapolated to give the corresponding contributions in 100% water and the slope of the change with eluent composition (Table 17). In many cases a polynomial function would have fitted the data points more accurately (as seen in Fig. 3) but the extrapolation of the polynomial would be very unreliable. The

values of τ_w obtained from the different organic modifiers are often significantly different, although in each case the relative magnitudes of the values are similar. The values for the phenyl group $\tau_w = 1.92$ and 1.47 from methanol and acetonitrile eluents, respectively, are very similar to the values obtained by Altomare *et al.*, $\tau_w = 2.01$ and 1.50 . However, although the corresponding values for the nitro group, $\tau_w = -0.180$ and -0.140 , were more similar for the two eluents they differed markedly from the values from the benzenesulphonamide study, $\tau_w = 0.31$ and 0.51 (Table 16). A similar more negative value was found for the benzene substitution for most of the hydrophilic groups, suggesting that the sulphonamide group had exerted an effect on the group contributions of these groups.

These problems with differences in the extrapolations to 100% water from the use of

TABLE 16

CALCULATED FUNCTIONAL GROUP CONTRIBUTIONS FOR WATER ELUENTS (τ_w) FOR SUBSTITUENTS ON BENZENESULPHONAMIDE

Calculated from methanol-water and acetonitrile-water separations. Based on data from Altomare *et al.* [57]. Conditions: column, μ Bondapak C₁₈.

Functional group	Group contribution (τ_w)			π	
	MeOH-water		MeCN-water, 4-	3-	4-
	3-	4-			
OC ₆ H ₁₃		2.99	2.27		2.55
Phenyl		2.01	1.50	0.55	1.93
OC ₄ H ₉	1.93	1.94	1.47	1.75	1.74
CH(CH ₃) ₂	1.41	1.45	1.09	1.35	1.40
I	1.05	1.09	1.03	1.27	1.24
Br	0.82	0.83	0.97	1.04	1.03
Cl	0.67	0.67	0.80	0.85	0.75
CH ₃	0.61	0.57	0.59	0.55	0.45
H	0.00	0.00	0.00		
OCH ₃	0.57	0.46	0.34	0.22	0.10
NO ₂	0.31	0.31	0.51	0.21	0.40
COCH ₃	0.53	0.50	0.46	-0.10	-0.11
CN	0.21	0.21	0.40	-0.07	-0.13
OH		-0.33	-0.22		-0.41
NH ₂	-0.42	-0.58	-0.59	-0.63	-0.97
CONH ₂	-0.14	-0.29	-0.39	-1.15	-1.14
SO ₂ NH ₂	-0.31	-0.50	-0.22	-0.81	-1.31
NHSO ₂ CH ₃		0.05	0.01		-0.71
NHCOCH ₃		0.29	0.24		-0.35

TABLE 17

CALCULATED FUNCTIONAL GROUP CONTRIBUTIONS IN WATER

Calculated by linear extrapolation of the τ values in Tables 2-4. $\tau = \tau_w + S \cdot$ (% organic modifier). S = Slope.

Functional group	Aqueous group contributions (τ_w)					
	Modifier					
	Methanol		Acetonitrile		THF	
	τ_w	S	τ_w	S	τ_w	S
Ph	1.924	-0.0185	1.475	-0.0153	1.143	-0.0179
Br	0.896	-0.0090	0.591	-0.0065	0.730	-0.0123
CH ₂ Br	0.644	-0.0060	0.578	-0.0065		
Cl	0.736	-0.0077	0.474	-0.0048	0.614	-0.0104
CH ₃	0.564	-0.0048	0.478	-0.0045	0.474	-0.0069
CH ₂ Cl	0.532	-0.0062	0.483	-0.0060		
F	0.132	-0.0021	0.130	-0.0020	0.162	-0.0033
H	0.000	0.0000	0.000	0.0000	0.000	0.0000
CO ₂ CH ₃	0.212	-0.0043	-0.083	-0.0001	-0.216	0.0001
OCH ₃	-0.006	-0.0003	0.072	-0.0014	-0.050	-0.0009
NO ₂	-0.180	-0.0001	-0.140	-0.0001	-0.110	-0.0013
COCH ₃	-0.284	-0.0003	-0.507	0.0040	-0.734	0.0073
CN	-0.446	0.0011	-0.402	0.0025	-0.562	0.0050
CH ₂ CN	-0.500	0.0011	-0.297	0.0008		
CHO	-0.576	0.0031	-0.403	0.0020	-0.760	0.0082
OH	-0.976	0.0055	-0.864	0.0058	-0.564	0.0039
NHCOCH ₃	-1.172	0.0087	-1.160	0.0090	-1.198	0.0099
CH ₂ OH	-1.100	0.0080	-1.144	0.0099		
NH ₂	-1.202	0.0084	-0.969	0.0084	-1.076	0.0126
CONH ₂	-1.314	0.0069	-1.619	0.0144	-1.738	0.0168
SO ₂ NH ₂	-1.942	0.0157	-1.304	0.0089	-1.428	0.0146

different eluent organic solvent were also found by Sherblom and Eganhouse [58], who reported $\log k'_0$ (calculated capacity factor in water) values for a series of alkylbenzenes, e.g. toluene, $\log k'_0 = 2.019$ from methanol and 1.936 from acetonitrile. When the corresponding group contributions functions for the methyl group are calculated, these were also found to be different ($\tau_w = 0.54$ from methanol and 0.29 from acetonitrile).

Although the details are not given here, the τ_w values and slopes of the change with eluent proportion were also calculated from the τ values (Tables 5-7) derived from the study by Schoenmakers *et al.* However, the correlations between the τ values and the eluent composition and the consequential τ_w values and S values are generally poorer but this may be because for

some groups a considerable extrapolation was required.

These relationships are similar to those developed by Schoenmakers *et al.* [29] in their study of the effect of structure and eluent composition on retention. They suggested that the slope of the relationship between capacity factors and solvent composition could be expressed as:

$$S = p + q \ln k_0 \quad (5)$$

where $\ln k_0$ is the capacity factor in 100% water as the eluent. This relationship held well for methanol-water and THF-water but not for acetonitrile-water. In contrast, as part of their study of solvophobic effects, Heron and Tchaplá

[16] also examined the relationship between S and $\log k'_0$ for a number of homologous series in methanol, acetonitrile and THF containing eluents and found close correlations in each case.

The same analysis can be applied to the data derived from the work of Smith and coworkers (Table 16). For each modifier there was a close correlation between the τ_w value and the slope (S) of the change with organic modifier (Fig. 6a-c) such that:

$$S = p + q\tau_w \quad (6)$$

$$\text{Methanol} \quad S = -0.00162 - \tau_w 0.0082 \quad (7)$$

$$\text{Acetonitrile} \quad S = -0.00106 - \tau_w 0.0090 \quad (8)$$

$$\text{THF} \quad S = -0.00214 - \tau_w 0.0118 \quad (9)$$

The value of q increased with the elution strength of the modifier so that THF the most non-polar eluent exerted the greatest effect on the slope. Unlike the study by Schoenmakers *et al.*, the correlations for the different modifiers were similar: methanol, $r = 0.9903$; acetonitrile, $r = 0.9942$; THF, $r = 0.9917$. These observations support the view that the retention mechanism is principally a partition process because as the proportion of organic modifier increased and the mobile phase became less polar, in each case, the effect of the hydrophobicity or hydrophilicity of the groups in the analyte decreased in magnitude.

In a recent study Belsner *et al.* [59] compared the $\log k'_w$ values and slopes in methanol eluents for homologous series of alkylbenzenes, alkyl aryl ketones and 4-alkylanilines and found a close relationship which they considered also reflected the lipophilicity of the analytes in a partition system.

Changes in the pH of the eluent can also effect relative retentions and the effective τ values of substituents if they cause partial or complete ionisation of the analytes. Some recent work has been reviewed by Hanai [6]. Ionised compounds are often separated by ion-pair chromatography and Riley *et al.* [11] reported that the τ values for substituents on analytes, which were separated by ion-pair chromatography, were unaffected by the concentration of ion-pair reagent

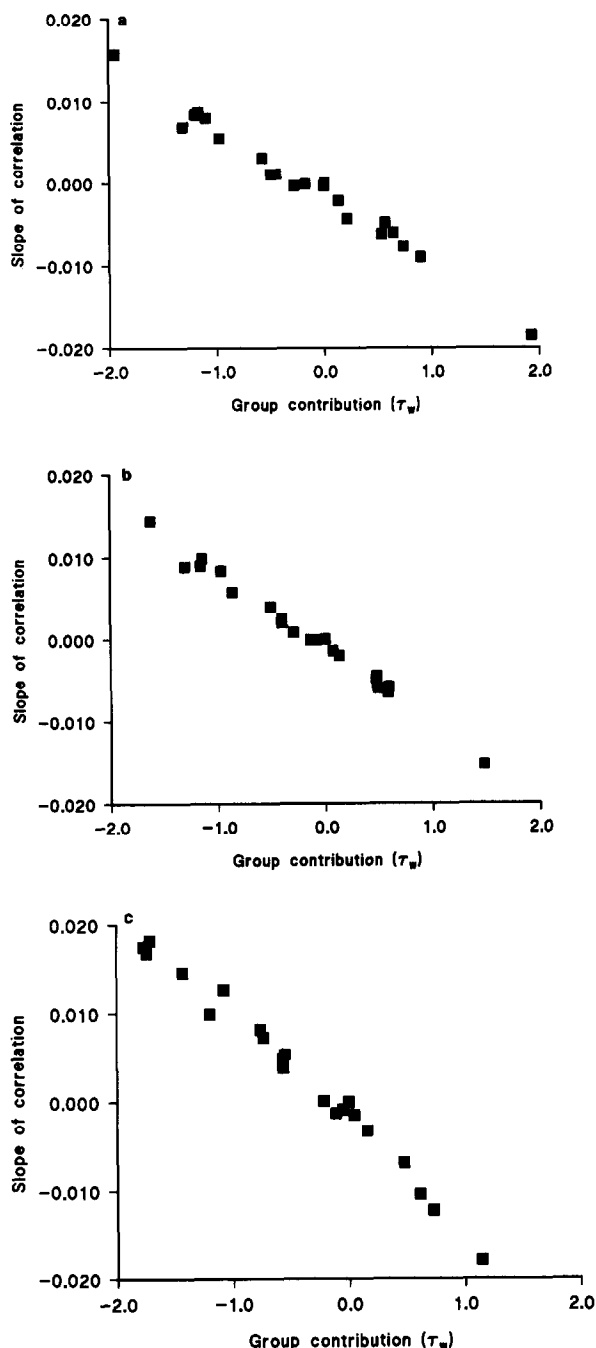


Fig. 6. Correlation between functional group contribution in 100% water (τ_w) and slope of relationship with proportion of organic modifier: (a) methanol; (b) acetonitrile; (c) THF.

or by the chain length of the reagent. The values decreased with increase in the proportion of methanol in the eluents. The change was linear

for neutral substituents but markedly curved for hydrogen-bonding groups.

The influence of the temperature of the eluent on τ values has been studied by Riley *et al.* [11] and by Lurie and Allen [60] and both studies found that systematic changes were present. For a consistent selectivity it is essential to control the column temperature.

2.4. Effect of stationary phases on τ values

Because there are such a wide range of differences in the capacity factors of analytes separated on different brands of ODS-bonded phases, it has been important to examine if there are also selectivity differences, which would result in different τ values.

Chen and Horváth [37] observed different τ values for phenolic, hydroxyl and methyl groups on the different octadecylsilyl (ODS)-bonded stationary phases, Partisil ODS, Spherisorb ODS and LiChrosorb ODS (Table 18). The *para*- and *meta*-phenolic groups were the most affected and had more negative τ values on the LiChrosorb-ODS column than the other columns.

Group contributions (Table 19) on a wide range of different stationary phases, which were calculated from unpublished capacity factors (provided by L.A. Witting) for a limited group of analytes, showed significant but often small differences between the group contributions for methyl, carbomethoxyl, nitro and acetyl hydroxyl groups. Changes in the phenolic hydroxyl

group were larger and the τ values on a LiChrosorb RP-18 column and particularly on a Zorbax ODS column were more negative than on the other columns. This last column material was also found to give a much lower retention index value for *p*-cresol (approximately $I = 739$ compared to 760) compared to other ODS phases in studies by Smith and co-workers [61,62]. However, in their study of sterols Di-Bussolo and Nes [48] compared the effects on two columns with C_{18} and C_8 bonded phases and C_{18} bonded columns of the same brand and found that the relative changes were constant for the C_{18} phases but differed for the C_8 phase although the two sets of data could be closely correlated.

As part of the studies on substituted flavanoids, Kahie *et al.* [63] determined group contribution values for a range of substituents on C_8 , phenyl and CN columns and compared them with earlier results on an ODS-silica column. In contrast to the earlier studies by Smith and Burr [12] and Wells and Clark [56], in most cases the $\Delta \log k'$ values were effectively independent of the composition over the ranges 50–70% or 40–60% methanol. A high correlation and a slope near unity between the contributions on the octyl and ODS columns ($C_{18}/C_8 = 1.07$) suggested that the mechanisms of interaction on the two columns were similar. The relationship between the contributions on the other columns differed ($C_{18}/\text{phenyl} = 0.63$; $C_{18}/\text{CN} = 0.68$ and between $\text{CN}/\text{phenyl} = 0.76$) with much poorer correla-

TABLE 18

COMPARISON OF FUNCTIONAL GROUP CONTRIBUTIONS DETERMINED ON DIFFERENT BRANDS OF ODS-SILICA

Data from Chen and Horváth [37]. Substituents on phenylethanes. Conditions: eluent, 0.1 M phosphate buffer pH 2.1.

Column	Group contribution (τ)			
	Group			
	<i>p</i> -OH	<i>m</i> -OH	1-OH	2-CH ₃
Partisil ODS	-0.537	-0.331	-0.856	0.390
Spherisorb ODS	-0.676	-0.390	-1.209	0.455
LiChrosorb ODS	-0.803	-0.522	-0.936	0.399

TABLE 19

COMPARISON OF FUNCTIONAL GROUP CONTRIBUTIONS DETERMINED ON DIFFERENT BRANDS OF ODS-SILICA

Based on unpublished capacity factors from L.A. Witting. Conditions: eluent, methanol-water (50:50).

Column	Group contribution (τ)				
	CH ₃	CO ₂ CH ₃	NO ₂	COCH ₃	OH
μ Bondapak C18	0.30	0.05	-0.10	-0.24	-0.59
Chromosorb LC-7	0.29	0.08	-0.13	-0.22	-0.58
LiChrosorb RP-18	0.33	-0.03	-0.24	-0.37	-0.72
Nucleosil C18	0.35	-0.01	-0.15	-0.31	-0.68
Partisil PKS-ODS	0.32	0.07	-0.15	-0.25	-0.58
Spherisorb ODS	0.29	0.13	0.08	-0.16	-0.59
Supelcosil LC-18	0.34	-0.04	-0.25	-0.38	-0.68
Zorbax BP-ODS	0.36	0.00	-0.24	-0.32	-0.94

tions suggesting that in these cases the mechanism of retention was different. Thus a different selectivity could be obtained by changing columns, which might enable pairs of compounds, which were unresolved on a C₁₈ column, to be resolved.

Although most reversed-phase chromatography is carried out using alkyl bonded-silica based stationary phases there has been recent interest in the application of polystyrene-divinylbenzene (PS-DVB) stationary phases because of their stability at extremes of pH and

TABLE 20

FUNCTIONAL GROUP CONTRIBUTIONS FOR SELECTED GROUPS ON DIFFERENT POLYSTYRENE DIVINYLBENZENE COLUMNS

Values calculated from Sun and Fritz [64]. Conditions: eluent, acetonitrile-water (50:50).

Functional group	Group contribution (τ)		
	Column		
	PS-DVB	PS-DVB-COCH ₃	PS-DVB-C(CH ₃) ₃
Ph	0.80	0.77	0.85
I	0.43	0.45	0.51
Br	0.37	0.43	0.44
Cl	0.26	0.32	0.32
CH ₃	0.21	0.14	0.25
F	-0.04	0.03	-0.02
H	0.00	0.00	0.00
CO ₂ CH ₃	-0.01	-0.13	-0.07
OCH ₃	0.02	-0.03	0.03
NO ₂	-0.09	-0.01	-0.08
COCH ₃	-0.34	-0.37	-0.35
OH	-0.72	-0.54	-0.82
CO ₂ H	-0.92	-0.26	-1.00
CH ₂ OH	-0.92	-0.72	-1.00

the absence of active silanols which can cause the tailing of bases. In a recent study, Sun and Fritz [64] have compared the capacity factors of a number of mono- and disubstituted aromatic compounds on a PS–DVB column with the retention on acetylated and *tert.*-butylated PS–DVB columns using acetonitrile–water (50:50) as the eluent. They expressed the effects of the different functional groups as capacity factors relative to that of benzene ($k'_{\text{Ph-X}}/k'_{\text{Ph-H}}$). Systematic differences in the increments were found between the three stationary phases and from these values the corresponding τ values have been calculated (Table 20). Comparison with the corresponding values on an ODS-silica column suggested that the contributions for the non-polar groups were more positive and for the polar groups more negative, which is in accord with observations by Smith and Garside [65] on comparing retention indices on ODS-Silica and PS–DVB columns. In addition, Sun and Fritz [64] determined the methylene increments of homologous alkylbenzenes and 4-alkylphenols. These were also found to differ on the different phases ($\log k'$ increment for alkylbenzenes: PS–DVB, 0.21; PS–C(CH₃)₃, 0.23; and PS–DVB–COCH₃, 0.18 and for the 4-alkylphenols: PS–DVB, 0.165; PS–C(CH₃)₃, 0.185; and PS–DVB–COCH₃, 0.15). In an earlier study Lurie and Allen examined fentanyl derivatives on ODS-silica and PS–DVB columns and compared τ values for substituents.

Because Haky and Vemulapalli [66] were concerned that the free silanol groups could cause specific interactions and that the limited pH stability range of silica based stationary phases could restrict the ability to carry out ion-suppression in studies on $\log P$ values, they examined the retentions of a wide range of aromatic analytes on octadecylsilyl bonded alumina, octadecylsilyl bonded silica, polybutadiene coated-alumina and an octadecyl-coated polystyrene–divinylbenzene (ACT-1) phases. From these results the corresponding group contributions can be calculated (Table 21). However, direct comparison is difficult as different mobile phases had to be used in each case to achieve similar capacity factors $k' = 4$ – 6 for benzene. The order of elution followed that

TABLE 21

FUNCTIONAL GROUP CONTRIBUTIONS DETERMINED ON DIFFERENT TYPES OF REVERSED-PHASE COLUMNS

Based on capacity factor data reported by Haky and Vemulapalli [66] for substituents on benzene. Conditions: eluent, methanol–pH 7.4 buffer.

Functional group	Group contribution (τ)			
	Stationary phase ^a			
	ODA	ODS	PBD	ACT-1
	Methanol (%) ^b			
	30	55 ^c	25	80
Ph-X				
Ph		0.89		
Br	0.61	0.41	0.68	0.46
CH ₃	0.41	0.21	0.43	0.25
CO ₂ CH ₂ CH ₃	0.43	0.28	0.24	0.25
Cl	0.48	0.34	0.53	0.07
CO ₂ CH ₃	0.09	–0.12	–0.12	0.25
OCH ₃	0.01	–0.15	–0.06	–0.05
H	0.00	0.00	0.00	0.00
COCH ₃	–0.33	–0.45		–0.42
CN	–0.40	–0.55	–0.70	–0.29
OH	–0.40	–0.75	–1.04	–0.77
CH ₂ OH	–0.54	–0.64	–1.15	–1.43
NHCOCH ₃	–0.71	–0.76	–1.16	
NH ₂	–0.69	–0.79	–1.21	–1.43
CONH ₂	–0.73	–1.01	–1.39	–0.84

^a Stationary phases: ODA = octadecylsilyl-coated alumina (experimental material); ODS = ODS-Econosil; PBD = polybutadiene-coated Unisphere alumina; ACT-1 = octadecyl-modified polystyrene–divinylbenzene.

^b Aqueous buffer: pH 7.4 phosphate except ODA which used a pH 7.4 4-morpholinopropanesulphonate (MOPS) buffer.

^c $\log k'$ benzene taken as 0.731 instead of 1.731 as published.

of the $\log P$ values and the ODS-coated columns gave similar results to those in Table 2 for the equivalent mobile phase composition. Even though the polybutadiene coated alumina column was very different in structure, it gave contributions which generally followed the trends observed on the ODS materials towards a greater magnitude of the τ values (more negative or more positive) as the proportion of methanol decreased. The group contributions on the ACT-

1 column followed this trend except that more negative values were found than on an ODS-silica column with the same eluent (Table 2) for the phenolic hydroxyl ($\tau = -0.77$; Table 2, $\tau = -0.50$), hydroxymethylene group ($\tau = -1.43$; Table 2, $\tau = -0.42$) and amino groups ($\tau = -1.43$; Table 2, -0.51). Although similar effects were not noted by Sun and Fritz (Table 20) using acetonitrile, Smith and Garside [65] found that with methanol containing eluents, hydroxyl containing compounds were eluted more rapidly, compared to less polar analytes, from PS-DVB columns than from ODS columns.

2.5. Comparison between τ values and Hansch π values

As has already been noted in many of the studies of functional group contributions, the τ values, or more often the τ_w values, have been compared with Hansch π constants, Rekker f constants, or other molecular descriptors and usually a close correlation is obtained. For example, in early work Hafkenschied and Tomlinson [67] examined the retention of a number of 1,4-disubstituted benzenes and measured the extrapolated τ_w values for combinations of methyl, chloro, nitro, amino and carboxylic acid groups using methanol-water eluents on an ODS-Hypersil column. These showed a close correlation with the corresponding π values. The τ_w values for the monosubstituted groups (methyl, 0.54; chloro, 0.66; nitro, -0.14 ; hydroxyl, -0.70 ; amino, -1.19 ; and carboxylic acid, -0.13) compared well with those obtained in other studies. 4-Nitrophenol and 4-nitroaniline showed large differences and the nitro group contributions were 0.40 and 0.45, respectively, with similar significant changes in the values of the hydroxyl and amino groups suggesting a strong interaction was present.

In order to obtain a comparison of retentions with octanol-water partition coefficients, Unger *et al.* [68] measured a number of $\log k'_w$ values directly by using an ODS column and a mobile phase of pH 7.0 buffer saturated with octanol. The corresponding τ_w values derived from the monofunctional analytes can be calculated

TABLE 22
AQUEOUS FUNCTIONAL GROUP CONTRIBUTIONS

Based on log capacity factors reported by Unger *et al.* [68]. Conditions: column, ODS-bonded silica plus octanol; eluent, octanol-saturated pH 7.0 buffer; temperature, 25°C.

Functional group	Group contribution (τ_w)
COPh	1.01
Cl	0.704
N(CH ₃) ₂	0.168
H	0.000
OCH ₃	-0.012
COCH ₃	-0.480
CN	-0.574
CH ₂ CN	-0.608
CHO	-0.606
OH	-0.653
NHCOCH ₃	-0.940
NH ₂	-1.200

(Table 22) and show a good comparison with the values obtained earlier.

Riley *et al.* [11] and Tomlinson *et al.* [19] found good correlations between τ values and π values for a range of substituents on phenylazapurines, phenyltriazines and benzoic acids, although the correlation was poorer if 2-substituents, which could undergo hydrogen bonding, were included [11]. The τ_w values obtained by Altomare *et al.* [57] for substituents on benzenesulphonamides correlated well ($r = 0.953$) with π values (Table 16). They found no significant differences between 3- or 4-substituents and suggested that no significant crossing interactions were taking place. Gago *et al.* [41] used the measured $\log k'$ values for 3- and 4-substituted pyridines to calculate $\log P$ values (given as $\log P^*$) and found that these correlated well with literature $\log P_{o/w}$ values suggesting that the τ values and group contributions to $\log P$ were closely related. Lurie and Allen [60] have examined τ values for methyl, alkyl and fluoro groups on fentanyl homologues and analogues and correlated the changes with calculated molecular connectivity values.

The values of the aqueous group contributions (τ_w , Table 17) from the three modifiers, calcu-

lated earlier from the retention data of Smith and co-workers, were compared with Hansch contributions π (Table 2). Despite the differences between the values from the different modifiers, there was a good overall correlation (Fig. 7, $r = 0.9710$). The values for group contributions (Tables 2-4) for individual eluents, which would give similar capacity factors, were also compared with the Hansch contributions (Fig. 8a-c). The correlations for the methanol-buffer (50:50) eluent were very good ($r = 0.9850$) but there were greater deviations for acetonitrile-buffer (40:60) ($r = 0.9645$) and THF-buffer (30:70) ($r = 0.9537$) and most of the changes seemed to be occurring between the more hydrophilic groups. In both acetonitrile and THF, the noticeable groups, which had a relatively more negative group contributions than other groups with similar π values were the carboxamide (CONH_2), N-acetyl (NHCOCH_3), hydroxyl (OH) and methylenehydroxyl (CH_2OH) groups. However, the amino and sulphonamide groups were apparently unaffected.

In the same way the group contributions for aliphatic substituents on ethylbenzene (Table 8) can be compared with the corresponding fragmental constants f (Fig. 9, $r = 0.9534$), which were chosen as they are considered to be a better guide to the properties of these groups than

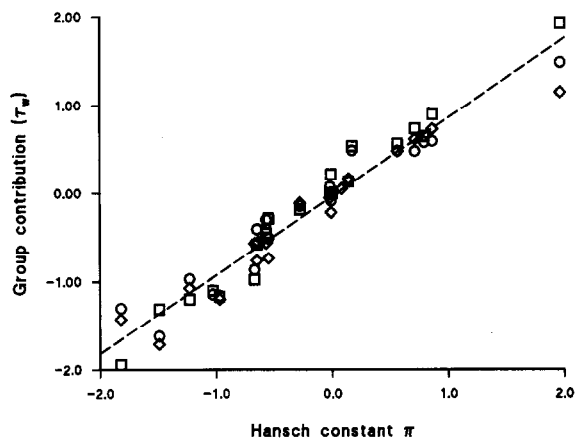


Fig. 7. Overall relationship between functional group contribution in water (τ_w) derived from three different organic modifiers and Hansch π constants. \square = Methanol; \circ = acetonitrile; \diamond = THF.

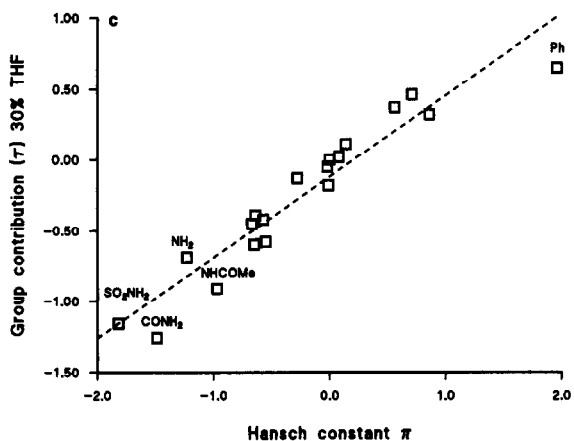
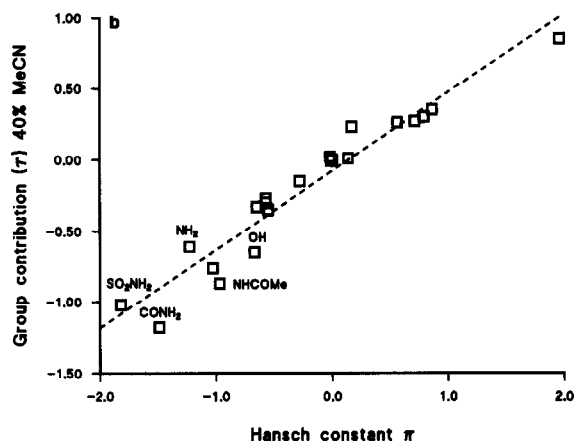
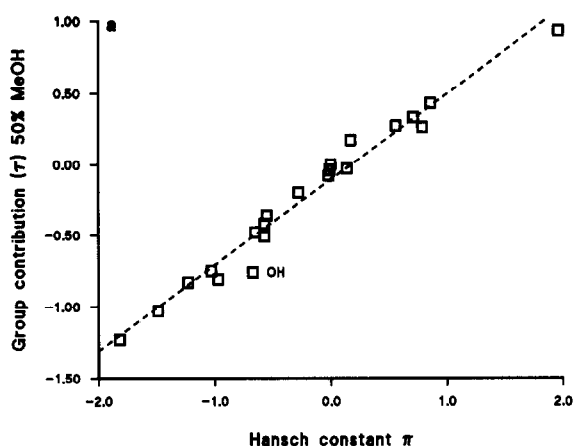


Fig. 8. Relationship between functional group contribution (τ) derived from three different organic modifiers and Hansch π constants. (a) Methanol; (b) acetonitrile; (c) THF.

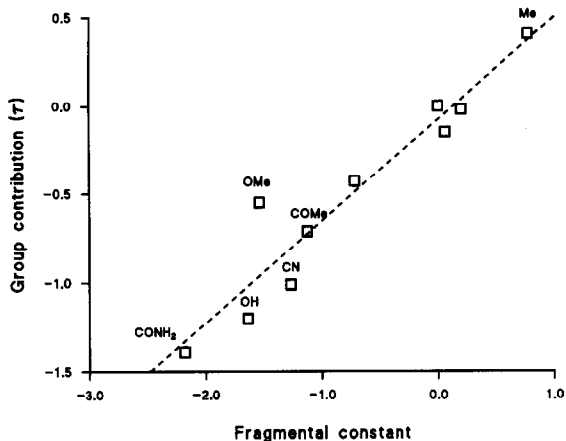


Fig. 9. Correlation of functional group contributions for aliphatic substituents and Rekker f constants. Data from Table 8 and ref. 21. Eluent; methanol–pH 7.0 buffer (40:60); correlation = 0.9282.

Hansch π values [21]. In this case the most noticeable outlier was the methoxyl group.

2.6. Retention effects in reversed-phase liquid chromatography

The correlations noted in the previous sections suggest that the retentions in reversed-phase separations are primarily governed by hydrophobicity effects, which closely reflect the octanol–water partition coefficients. Although often significant, the differences between modifiers have a relatively small influence, however, this is often sufficient to enable a considerable selectivity to be achieved in the separation of pairs of analytes, if one contains a group which is more sensitive to eluent differences. Thus the mobile phase must play an active part in the selection process.

Further confirmation of a partition mechanism comes from the reduction in the magnitude of absolute values of the group contributions with increasing proportions of the organic modifier. Both hydrophobic and hydrophilic groups show equivalent effects as the polarity of the eluent decreases suggesting that they are retained by a similar mechanism. Similar views have been expressed in a recent paper by Kaliszan *et al.* [69] who have examined the structural features which contribute to hydrophobicity. They sug-

gested that the retention of a series of benzodiazepines was primarily related to the hydrophobicities of individual molecular regions and that at a first approximation the fragmental hydrophobicity parameters can be replaced by their simple arithmetical sum, rather than requiring a specific correction factor. They also considered that a linear change in $\log k'$ with organic modifier concentration was indicative of an absence of silanophilic interactions and should enable chromatographic measures of the hydrophobicity of analytes.

2.7. Other applications of τ values

Although this review concentrates on the application of group contributions in reversed-phase chromatography, the concept has also found application in other liquid chromatographic modes. Law [70] examined the effect of substituents in a series of monofunctional aryl alkyl amines, which were chromatographed in a cation exchange mechanism on a silica column using methanol high-pH eluents. He determined τ values for N-methyl, methyl and hydroxyl substituents on the original compounds and their metabolites and suggested that the resulting contributions would be useful in predicting the retentions of metabolites from their parent compounds.

3. RETENTION PREDICTION USING FUNCTIONAL GROUP CONTRIBUTIONS

3.1. Prediction of the effect of different functional groups

From the above discussion, the additive nature of functional group contributions suggests that the τ values such as those presented earlier could be used to calculate the change in retention resulting from the introduction of a particular functional group into a parent compound. However, for these values to be valuable for general prediction, they need to be independent of a particular combination of column and eluent. A collation of the τ values reported in this survey for 100% water (τ_w) and for modifier–aqueous (50:50) eluents (Table 23) confirms that in most cases relatively similar values have been ob-

TABLE 23

SUMMARY OF FUNCTIONAL GROUP CONTRIBUTIONS FOR AROMATIC SUBSTITUENTS ON ODS-SILICA COLUMNS

Excludes polyfunctional and heterocyclic compounds. τ_w for a particular table are mean values from different solvents (or stationary phases). Values in parentheses (τ) for 55% organic modifier quoted when 50% not available.

Functional group	Functional group contribution (τ)				Hansch constant (π) [21]	Reference	
	Eluent					Table	Ref.
	Water (τ_w)	50% MeOH	50% MeCN	50% THF			
OPh	1.96	0.80 (0.90)			2.08	5	
Ph	1.54	0.93	0.64	0.21	1.96	2-4, 17	
	1.46	0.87 (0.89) (0.94) (0.95)	0.61	0.25		5-7 21 10	
C ₃ H ₇ (CH ₂) ₄	1.86		0.33	0.13	1.55	10	
			(0.60) (0.61)				6,7 10
OC ₃ H ₇ COPh	0.74 1.01	0.51	0.28	-0.06	1.05 1.05	10 5-7	
						22 10	
CH ₂ CH ₃ Br	0.91 0.74	0.54	0.34	0.26	1.02	5-7	
		0.43 (0.41) (0.39)	0.27	0.09		0.86	2-4,17 21 10
CH ₂ Br Cl	0.61 0.61 0.60 0.70	0.26	0.22		0.79 0.71	2,3,17	
		0.33	0.21	0.07		2-4,17	
		0.37	0.22	0.09		5-7	
		0.31 (0.34) (0.31)				15 22 21 10	
CH ₂	1.32	0.35 (0.23)	0.21		0.66		22 23
NHC ₃ H ₇ CH ₃	0.50 0.49	0.27	0.26	0.12	0.62 0.56	10	
		0.36	0.22	0.15		2-4,17	
		0.30 0.32 (0.21) (0.33)				15 19 21 10	
		(0.28) (0.24) (0.23)				21 10	
CO ₂ CH ₂ CH ₃					0.51	21	
						10	
OC ₂ H ₅ N(CH ₃) ₂	0.46 0.17	0.22		-0.64	0.38	10	
				0.12	0.18	5,7 22	
CH ₂ Cl	0.51	0.17	0.16		0.17	2,3,17	
F	0.14	-0.03	0.05	-0.01	0.14	2-4,17	
NHCH ₂ CH ₃				-0.03	0.08	6	
COC ₂ H ₅		(-0.08)		0.06	0.06	10	

(Continued on p. 412)

TABLE 23 (continued)

Functional group	Functional group contribution (τ)				Hansch constant (π) [21]	Reference	
	Eluent					Table	Ref.
	Water (τ_w)	50% MeOH	50% MeCN	50% THF			
H	0.00	0.00	0.00	0.00	0.00		
CH ₂ OCOCH ₃		(-0.08)				10	
CH=CHCN		(-0.21)				10	
OCOCH ₃		(-0.35)				10	
CO ₂ CH ₃	-0.03	-0.04	-0.09	-0.21	-0.01	2-4,17	
	0.25	-0.01	-0.08			5-6	
		0.01				15	
		0.03				19	
		(-0.12)				21	
		(-0.04)				10	
OCH ₃	0.01	-0.08	0.00	-0.10	-0.02	2-4,17	
	0.17	0.03	-0.02	-0.11		5-7	
		-0.02				15	
	-0.01					22	
		(-0.15)				21	
		(-0.02)				10	
CH=CHOH		(-0.30)			-0.17	10	
NO ₂	-0.14	-0.20	-0.15	-0.18	-0.28	2-4,17	
	-0.13	-0.20	-0.10	-0.19		5-7	
		-0.19				15	
		-0.15				19	
NHCH ₃	-0.07	-0.17		-0.06	-0.47	5,7	
		(-0.35)				10	
COCH ₃	-0.51	-0.36	-0.31	-0.36	-0.55	2-4,17	
	-0.39	-0.28	-0.29	-0.42		5-7	
		-0.30				15	
		-0.28				19	
	-0.48					22	
		(-0.45)				21	
		(-0.38)				10	
CN	-0.47	-0.42	-0.27	-0.30	-0.57	2-4,17	
	-0.39	-0.47	-0.25	-0.36		5-7	
		-0.33				15	
	-0.57					22	
		(-0.55)				21	
		(-0.47)				10	
CH ₂ CN	-0.40	-0.51	-0.25		-0.57	2,3,17	
	-0.61					22	
		(-0.50)				10	
CHO	-0.58	-0.48	-0.28	-0.34	-0.65	2-4,17	
	-0.61					22	
		-0.39				15	
OH	-0.80	-0.76	-0.54	-0.36	-0.67	2-4,17	
	-0.54					18	
	-0.65					22	
		-0.52				15	
		(-0.75)				21	

TABLE 23 (continued)

Functional group	Functional group contribution (τ)				Hansch constant (π) [21]	Reference	
	Eluent					Table	Ref.
	Water (τ_w)	50% MeOH	50% MeCN	50% THF			
NHCOCH ₃	-0.94				-0.97	22	
		(-0.72)				10	
		(-0.76)				21	
NHCHO		(-0.77)			-0.98	10	
CH ₂ OH	-1.12	-0.75	-0.63		-1.03	2,3,17	
	-0.84	-0.58	-0.72	-0.65		5-7	
		-0.52				15	
		(-0.64)				21	
		(-0.68)				10	
NH ₂	1.08	-0.83	-0.53	-0.43	-1.23	2-4,17	
	-0.70	-0.75		-0.37		5,7	
	-1.20					22	
		(-0.79)				21	
		(-0.80)				10	
CONHCH ₃				-0.86	-1.27	4	
CSNH ₂		(-0.75)				10	
CONH ₂	-1.56	-1.03	-0.90	-0.84	-1.49	2-4,17	
		-0.95				15	
		(-1.01)				21	
		(-1.09)				10	
CON(CH ₃) ₂				-0.86			
SO ₂ NH ₂	-1.56	-1.23	-0.78	-0.67	-1.82	2-4,17	
(CH ₂) ₂ OH	-0.55	-0.47	-0.59	-0.51		6,7	
(CH ₂) ₃ OH	-0.07	-0.13	-0.42	-0.51		6,7	

tained from different studies and this agrees with the earlier comparison of methylene contributions [22]. In some cases, only values in modifier-water (55:45) were available but these were very similar. Overall across a wide range of substituent groups there is also a general correlation between the τ values and the corresponding π values.

The relationships determined earlier between τ_w and S (eqns. 7–9) could be used to calculate approximate values of τ for specific eluent compositions using eqn. 10.

$$\tau = \tau_w + S \text{ B\%} \quad (\text{B\%} = \% \text{ organic modifier}) \quad (10)$$

$$\tau_{\text{MeOH}} = \tau_w + (-0.00162 - \tau_w 0.0082) \% \text{B} \quad (11)$$

$$\tau_{\text{MeCN}} = \tau_w + (-0.00106 - \tau_w 0.0090) \% \text{B} \quad (12)$$

$$\tau_{\text{THF}} = \tau_w + (-0.00214 - \tau_w 0.0118) \% \text{B} \quad (13)$$

For example, for the aldehyde group ($\tau_w = -0.58$) the calculated values can be compared with experimental values from Table 23: methanol-water (50:50), $\tau_{\text{calc}} = -0.42$ and $\tau_{\text{expt}} = -0.48$; acetonitrile-water (50:50), $\tau_{\text{calc}} = -0.37$ and $\tau_{\text{expt}} = -0.28$; THF-water (50:50), $\tau_{\text{calc}} = -0.35$ and $\tau_{\text{expt}} = -0.34$). The τ values calculated from eqns. 11–13 will probably be inaccurate outside the range of compositions used to calculate the original values, particularly at high modifier concentrations.

However, although these equations will reflect the different elution strengths of the different organic modifiers, they cannot reflect the specific selectivity changes resulting from modifier-analyte interactions as they are derived from a common set of τ_w values. The values will also ignore any differences that occur between different ODS-phases, although both these effects are relatively small compared to the influence of

eluent composition. Similar equations could also be derived for aliphatic groups although the number of available models is severely limited.

The principal limitation on the use of these correlations is that they can only be applied with any success when the introduced functional group is isolated and will not interact either electronically, sterically or by hydrogen bonding with any other groups or heteroatom that is present in the parent compounds.

3.2. Prediction of the retention of multifunctional compounds

So few there are few model systems for the effects of interaction between functional groups on the retention of bi- and polysubstituted analytes. For some compounds with non-interactive groups, a summation of contributions (τ) calculated for the appropriate eluent will probably give a good approximation. However, as was seen earlier the contributions of many groups can be seriously affected by the presence of a second substituent. The studies examined earlier (section 2.2.2) suggest some methods that can be used to predict the extent of these interactions, principally by the use of interaction parameters based on Hammett functions. However, specific values of the parameters will be needed to be determined, which reflect the effect of the different organic modifiers. Additional terms will be required for the often large effects of hydrogen bonding.

In addition when substitution occurs on a heterocyclic aromatic ring the influence of the hetero atom depends both on the nature and position of the substituents. As seen earlier with furans and pyridines the apparent group contribution can differ from that on an aromatic ring (Tables 13 and 14).

Thus for many analytes the predicted retention values obtained for substitution onto an already substituted parent will be only approximate and at the present state of the understanding will have limited value in method development. In the future a close collaboration between QSAR and QSRR studies will be needed to develop a better model of the partitioning properties of analytes.

4. CONCLUSIONS

Functional group contributions (τ values) have been derived for a wide range of substituent groups on a range of parent structures. The frequent close correlations between both the τ_w and τ values in different eluents and the Hansch π constants, suggest that the principal mechanism of retention in reversed-phase chromatography is a partition process, which is closely related to the octanol–water distribution and that both size and polarity of the carbon skeleton and functional groups play a part.

Generally the magnitudes of the τ values decrease with increased proportion of modifier in the eluent as hydrophobic effects become less important. There are differences in the τ values determined with different modifiers reflecting selectivity changes between the eluents. Changes in the brand of C_{18} stationary phases generally cause small differences in the τ values but different types of bonded-phase and different phase materials, such as polymeric phases, can have a more marked effect.

The τ values can be used to predict the retention of simple substituted compounds based on a parent structure. Problems arise with polyfunctional compounds when interaction between substituents can markedly alter the group contribution because of electronic, steric or hydrogen bonding effects.

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