Journal of Chromatography, 185 (1979) 197–224

CHROM. 11,999

FUNCTIONAL GROUP BEHAVIOUR IN ION-PAIR REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING SURFACE-ACTIVE PAIRING IONS

C. M. RILEY, E. TOMLINSON* and T. M. JEFFERIES

School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath, Avon BA2 7AY (Great Britain)

SUMMARY

The behaviour of functional groups having widely differing physico-chemical character has been examined in ion-pair reversed-phase high-performance liquid chromatography using surface active pairing ions. The effects of temperature, mobile phase, organic modifier type and percent composition, stationary phase carbon loading and type, ionic strength, and pairing ion structure, charge and concentration on extra-thermodynamic functional group contribution values, have all been determined. Analysis of group behaviour within the framework provided by solvophobic theory is often found to be possible using linear free-energy relationship approaches, and it is shown that retention behaviour can be described as ion-pair formation in the mobile phase followed by distribution to the stationary phase. In addition, substituent behaviour is found to exhibit linear enthalpy–entropy compensation behaviour, suggesting further that a common retention mechanism can be described for all ionized solutes using these pairing ions.

INTRODUCTION

Many compounds of pharmaceutical or biochemical interest, (for example pharmacons, metabolites and phytochemicals), are either polar or can be found in the ionised form. The use of ion-pair high-performance liquid chromatography (HPLC) to analyse solutes of this type is now well established[1]. Such techniques involve the addition of an oppositely charged pairing ion to the chromatographic phase system, so that solute retention is effected by ion pairing via various mechanisms. Ion-pair reversed-phase (RP) HPLC, where the pairing ion is added to the mobile phase, has been shown to have high flexibility in retention and selectivity control, particularly when the pairing ion is surface active[2,3]. The area of ion-pair HPLC has recently been reviewed[4].

* To whom correspondence should be addressed. Present address: Subfaculty of Pharmacy, University of Amsterdam, Plantage Muidergracht 24, Amsterdam, The Netherlands.

Use of surface active agents as pairing ions in RP-HPLC is intriguing, both theoretically and practically. They nearly always result in improved solute resolution and column efficiency over other types of pairing ions, and they are often the pairing ion of choice[4]. Theoretically it has been argued that these large hydrophobic ions exert their action either via a dynamic ion-exchange event[5–7], or via ion pairing in the mobile phase followed by distribution to the stationary support[2,8], or via a combination of both effects[8].

In this paper a semi-empirical approach for rationalizing solute retention in ion-pair surfactant RP-HPLC is presented, and is comprised of a thorough study of functional group contributions with respect to various environmental and constitutional factors. Anionic and cationic pairing ions have been used, and the effect of temperature, organic modifier type and percent composition, stationary phase carbon loading and type, pairing ion concentration and hydrophobicity, and ionic strength on functional group behaviour, have been determined in detail.

Extra-thermodynamic approaches to data analysis have been used wherever possible, including examinations of linear free-energy relationships and enthalpy–entropy compensation effects.

EXPERIMENTAL

Apparatus

HPLC systems were custom built and have been described previously[9,10]. Column and injection valve temperature were controlled, ($\pm$ 0.2°C), by enclosure in a modified gas chromatographic oven (Perkin-Elmer F11), or, for the thermodynamic study, by immersion in a thermostatically controlled ($\pm$ 0.1°C) water bath.

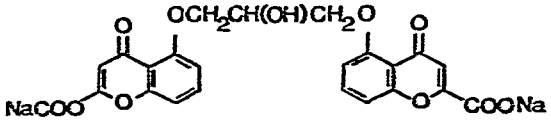
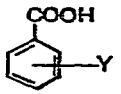
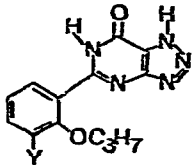
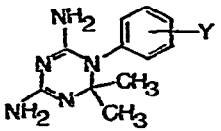
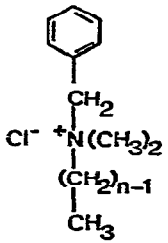
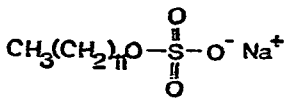
Materials

Scheme I gives structures of solutes and pairing ions used in this study. Alkylbenzyldimethylammonium chlorides were as described previously[10], except for the C_{14} and C_{16} homologues which were of "puriss" grade and were supplied by Fluorochem (Glossop, Great Britain). Alkylsulphates[9] were supplied as pure (> 98%) by Cambrian Chemicals (Croydon, Great Britain) except for sodium dodecylsulphate which was supplied by BDH (Poole, Great Britain), and was described as specially purified for biochemical work. Sodium cromoglycate was as described previously[10], substituted 8-aza-purin-6-ones and 1,3,5-*s*-triazines were kindly donated by Dr. K. R. H. Wooldridge (May and Baker, Dagenham, Great Britain), and were used as received. Substituted benzoic, phenylacetic and cinnamic acids were of at least reagent grade and were obtained from BDH, Cambrian Chemicals, and Fisons (Loughborough, Great Britain). All other chemicals were of AnalaR grade (Fisons), except for acetonitrile, *n*-hexane and methanol which were of HPLC grade (Rathburn, Great Britain). Water was double distilled from an all-glass still, except for the study of ionic strength effects where it was also deionized.

Packing materials used were Spherisorb S5 ODS and Hexyl, both 5 μm, (Phase Separations, Queensferry, Great Britain); Partisil 10 ODS and Partisil 10 ODS-2 both 10 μm (Whatman, Maidstone, Great Britain); and ODS Hypersil, 5 μm (Shandon Southern Products, Runcorn, Great Britain).

SCHEME I

STRUCTURES OF SOLUTES AND PAIRING IONS

Compound	*Structure*	*Substituents*
Solute		
Sodium cromoglycate		
Benzoic acids		Y=2;3;4-NH_2 2;3;4-NO_2 2;3;4-OH 2;3;4-CH_3
8-Aza-purine-6-ones		Y=H;SO_2NH_2;NH_2;CN;OH; $SO_2C_3H_7$;SH;$CO_2C_2H_5$; OC_4H_9;$C(CH_3)_3$
1,3,5-*s*-Triazines		3-Y=*n*-C_6H_{13};$C(CH_3)_3$;$N(CH_3)_2$; SCH_3;CF_3;Br;CN;SO_2CH_3;OH $-(CH_2)_4$-phenyl;$-(CH_2)_4$-phenyl-4-OCH_3 $-O$-*n*-C_6H_{13};$-O$-*n*-C_9H_{19} 4-Y=*n*-C_6H_{13};SCH_3;CF_3;Br;$NHCOCH_3$; CN;OH;SO_2CH_3;SO_2NH_2;$C(CH_3)_3$
Pairing ions		
Alkylbenzyldimethyl-ammonium chlorides		*n*=8–14
Sodium dodecyl-sulphate		

Procedures

Column packing and preparation methods and chromatographic procedures were as described previously[10]. Functional group contributions were derived from at least triplicate determinations of capacity ratios. Mobile phase pH was adjusted so that solutes being studied were in the fully ionised state.

Non-linear least squares analysis was carried out using a PDP-11 minicomputer using a standard program employing Marquardt's gradient-expansion method.

RESULTS AND DISCUSSION

Functional group values

Functional group contribution towards retention may be defined as

$$\tau = \log r_{ji} = \log(\kappa_j/\kappa_i) \quad (1)$$

where κ are capacity ratios of solutes j and i which differ by a functional group, r is the selectivity coefficient and τ is its logarithmic form. In this present study the reference solute i is taken as the unsubstituted analogue. The capacity ratio may be related to solute distribution coefficients (K_D) by

$$K_D = \kappa/\varphi \quad (2)$$

where φ is the phase volume ratio. Hence the group contribution term, τ (or $\log r_{ji}$), is analogous to other substituent extra-thermodynamic terms[11] such as the ΔR_M[12] term in thin-layer chromatography (TLC), the Hansch π[13] term in liquid–liquid distribution, or the Hammett electronic σ[14] term. It is an extremely convenient function with which to rationalise the factors affecting solute retention since it is independent of phase volume ratio, as experimentally verified for ion-pair systems by Wahlund and co-workers[15,16]. Accordingly, we present in this communication data which show how τ is affected by various environmental and constitutional factors. Wherever possible, the data has been rationalised by means of linear free-energy relationships[17]. Group values determined under standard sets of condition for three substituted solute series are given in Table I. All group values are correlated well with π values indicating that the physico-chemical phenomena underlying liquid–liquid distribution between bulk phases are also controlling retention in ion-pair LC systems. The appropriate correlation equations (analysed by linear least squares regression) are given below, *i.e.*

benzoic acids:	$\tau = 0.49\pi + 0.03$	$n = 10$	$r = 0.940$	(3)
azapurines:	$\tau = 0.49\pi - 0.01$	$n = 10$	$r = 0.983$	(4)
triazines:	$\tau = 0.51\pi - 0.05$	$n = 20$	$r = 0.961$	(5)
all series:	$\tau = 0.49\pi - 0.02$	$n = 40$	$r = 0.965$	(6a)
	$\tau = 0.47\pi - 0.002$	$n = 44$	$r = 0.941$	(6b)

where n and r refer to the number of data points and the correlation coefficient, respectively. π values (water–1-octanol) have been obtained from refs. 13, 18 and 19 for the substituted benzoic acid series, from ref. 19 for the substituted azapurines, and from refs. 19 and 20 for the triazine series. Eqn. 3 is for 3- and 4-substituents, whereas eqn. 6b includes the 2-substituted benzoic acid groups. Regressed slope coefficients for eqns. 3–6 are < 1 reflecting the presence of methanol in the chromatographic mobile phase[21]. Other workers[22,23] have described similar linear free-energy relationships between bulk phase partition parameters and ion-pair HPLC data (see Fig. 4, ref. 4). Fig. 1 gives the general relationship between π and τ values (eqn. 6) found for a variety of pairing ions of both negative and positive charge,

TABLE I

FUNCTIONAL GROUP CONTRIBUTION VALUES FOR AROMATIC SUBSTITUENTS

Solute class					
*Triazines**		*Azapurines***		*Benzoic acids****	
Function	τ	*Function*	τ	*Function*	τ
				2-OH	0.25
3-OH	−0.37	3-OH	−0.19	3-OH	−0.30
4-OH	−0.42			4-OH	−0.45
4-$NHCOCH_3$	−0.30			2-NH_2	−0.11
		3-NH_2	−0.48	3-NH_2	−0.50
3-$N(CH_3)_2$	−0.50			4-NH_2	−0.55
3-SO_2CH_3	−0.36	3-SO_2CH_3	−0.71	2-NO_2	−0.14
4-SO_2CH_3	−0.45			3-NO_2	0.16
				4-NO_2	0.15
4-SO_2NH_2	−0.69	4-SO_2NH_2	−0.71		
3-O-n-C_6H_{13}	1.20	3-$SO_2C_3H_7$	−0.17	2-Cl	0.03
				3-Cl	0.49
3-CN	−0.29	3-CN	−0.31	4-Cl	0.45
4-CN	−0.33				
				2-CH_3	0.05
3-$C(CH_3)_3$	0.90	3-$C(CH_3)_3$	0.95	3-CH_3	0.24
4-$C(CH_3)_3$	0.95			4-CH_3	0.21
3-n-C_6H_{13}	1.77	3-OC_4H_9	0.71	CH_2§	0.05
4-n-C_6H_{13}	1.80	3-$CO_2C_2H_5$	0.30	CH=CH§§	0.31
3-SCH_3	0.38	3-SH	0.04		$\tau_{4/2}$§§§
4-SCH_3	0.29			OH	−0.70
				NH_2	−0.44
3-CF_3	0.48			NO_2	0.29
4-CF_3	0.23			Cl	0.42
				CH_3	0.16
3-Br	0.30				
4-Br	0.20				

* Stationary phase Spherisorb ODS; mobile phase methanol–water (1:1), $2.6 \cdot 10^{-4}$ mol·dm^{-3} sodium dodecylsulphate; flow-rate 1.7 ml·min^{-1}; 30°C; pH 2.2 (0.1% H_2SO_4).

** Stationary phase Spherisorb ODS; mobile phase methanol–water (1:1), $1.0 \cdot 10^{-4}$ mol·dm^{-3} undecylbenzyldimethylammonium chloride; flow-rate 2.0 ml·min^{-1}; 30°C; pH 7.5 ($2.5 \cdot 10^{-2}$ mol·dm^{-3} K_2HPO_4).

*** Stationary phase Spherisorb ODS; mobile phase methanol–water (1:1), $4.0 \cdot 10^{-4}$ mol·dm^{-3} terdecylbenzyldimethylammonium chloride; flow-rate 1.5 ml·min^{-1}; 30°C; pH 7.5 ($2.5 \cdot 10^{-2}$ mol·dm^{-3} K_2HPO_4).

§ Phenylacetic acid.

§§ Cinnamic acid.

§§§ Eqn. 8.

various solute series and mobile phases of varying compositions and acid strengths. This correlation agrees with the conclusions of Horváth *et al.*[8], that a solvophobic effect[24] is primarily responsible for retention in ion-pair systems (see below), as is shown by linear relations between retention behaviour and solute (or group) hydrocarbonaceous surface areas[25] which are directly related to hydrophobicity parameters.

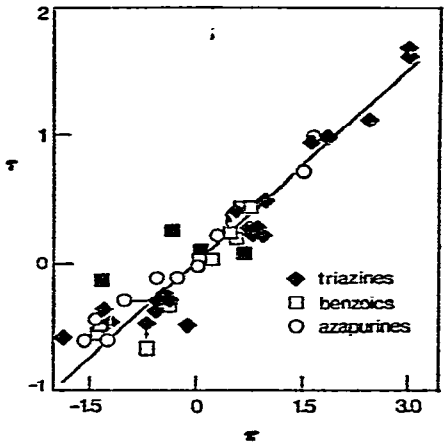


Fig. 1. Relationship between liquid–liquid distribution group values, π[19] and chromatographic group values, τ, derived from three solute series using chromatographic conditions given in Table I (see eqn. 6). Closed data points for the benzoic acids correspond to *ortho* substituents using π values calculated from toluene and phenoxyacetic acid series.

It has been argued[25] that LC can provide hydrophobicity parameters for use in quantitative structure-biological activity models. It is found here, (eqn. 7) that τ values determined in an ion-pair RP-HPLC system using sodium dodecylsulphate as the pairing ion and methanol as the organic modifier (Table I), can be used as indices of hydrophobicity for relating the physico-chemical properties of a series of 1,3,5-*s*-triazines to their minimum inhibitory concentrations (MIC)[20] against *Staphylococcus aureus*, *i.e.*

$$\log(1/\mathrm{MIC}) = 1.0\tau + 2.9 \qquad n = 10 \qquad r = 0.965 \tag{7}$$

The group contribution approach can be extended to indicate variation in retention between steric isomers, hence, for example, we may write:

$$\tau_{4/2} = \log(\kappa_{para}/\kappa_{ortho}) \tag{8}$$

where $\tau_{4/2}$ relates to the differences in group selectivity substituted in the *ortho* position compared to the *para* ring position. Appropriate values are included in Table I, and since they are analogous to ΔR_M[12] steric terms should be of similar use in HPLC.

Concentration of pairing ion

It has been demonstrated experimentally[3,5,10] that surface active pairing ions adsorb onto the stationary phase in RP systems, although calculations[3,10] show that surface coverage is very low (1–5%). Fig. 2 shows the effect of pairing ion concentration on the capacity ratios of a number of substituted benzoic acids, the relationship is a complex one with an initial sigmoidal dependence of the capacity ratios upon pairing ion concentration followed by a fall in capacity ratio at higher concentrations. Horváth *et al.*[8] have shown that retention behaviour in ion-pair systems can be described by

$$\kappa = (\kappa' + B[X])\cdot(1 + K_1[X])^{-1}\cdot(1 + K_2[X])^{-1} \tag{9}$$

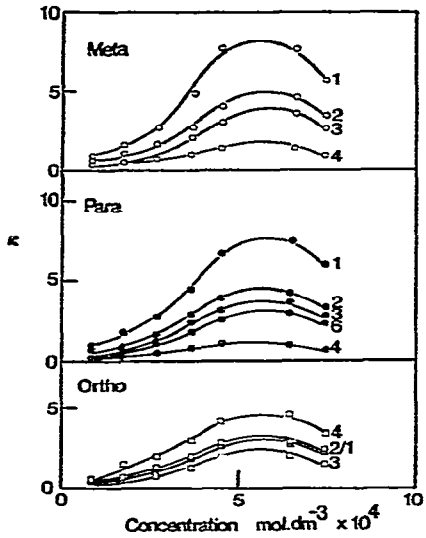


Fig. 2. Effect of pairing ion (terdecylbenzyldimethylammonium chloride) concentration on benzoic acid capacity ratios, κ, under the conditions given in Table I. Key: compounds 1–5, refer to chloro, methyl, nitro, hydroxy and amino substituents, respectively, and 6 to the unsubstituted molecule; 2-, 3- and 4-substituents are denoted by open squares, open circles and closed circles, respectively.

where $[X]$ is the pairing ion concentration, κ' is solute capacity ratio in the absence of pairing ion, K_1 is the ion-pair formation constant, K_2 is the pairing ion binding constant (with the stationary phase) and the meaning of B depends on the underlying physico-chemical equilibria controlling retention, such that for ion-pair formation in the mobile phase followed by distribution to the stationary phase $B = K_1K_3$, where K_3 is the ion-pair distribution constant, and for dynamic ion-exchange mechanisms $B = K_2K_4$, where K_4 is the formation constant for the solute–adsorbed pairing ion complex. Eqn. 9 is in the form of a parabolic dependency for κ on $[X]$ provided $K_1^{-1} > (K_2[X])^{-1}$. The initial sigmoidal effect shown by Fig. 2 has been argued[8] as due to initial pairing ion depletion at very low concentrations, and hence is related to solute concentration and column load capacity[27]. Pairing ion depletion causes the model described by eqn. 9 to be inappropriate at these low concentrations. Fig. 3 shows that group contribution towards retention is independent of pairing ion concentration in the present systems above 2×10^{-4} mol·dm^{-3}, indicating (a) a single retention mechanism and (b) that retention can be altered independently of selectivity by changing pairing ion concentration. A number of other studies have demonstrated that pairing ion concentration can cause complex changes in solute capacity ratio, and in retrospect these also show that although selectivity is often unchanged[5,8,28–34] with change in concentration, with some pairing ions (notably perchlorate[35]) selectivity can be concentration dependent. Since it can be demonstrated[4,10] that an increase in alkylbenzyldimethylammonium chloride concentration reduces column plate height and increases retention, it follows from the above that an increase in concentration will cause an increase in column resolution.

Recently[8], convincing arguments have been presented based on solvophobic theory that for normal alkylsulphates and sulphonates a dependence of $\log(B/\kappa' \cdot K_1)$

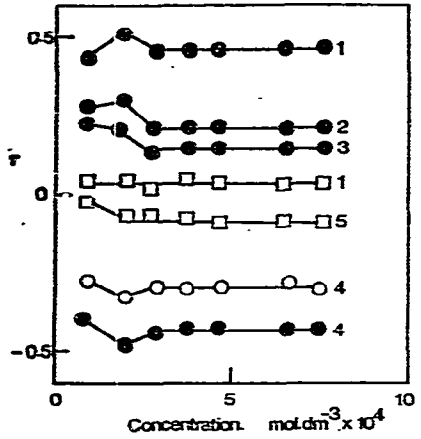


Fig. 3. Relationships between pairing ion concentration and τ for benzoic acids using same conditions as for Fig. 2. Plot shows constant τ values above *ca.* $2 \cdot 10^{-4}$ mol·dm^{-3} pairing ion concentration. (Note: data points for 3-chloro, 3-methyl and 3-nitro coincide with those of the corresponding 4-isomer and are not included.) Key as for Fig. 2.

on pairing ion chain length, (*i.e.* an indicator of hydrocarbonaceous surface area), is indicative of solute retention proceeding primarily by the formation of ion pairs in the mobile phase. Assuming this to be the case in this present study, we have analysed the data given in Fig. 2 [omitting the initial κ values determined at very low pairing ion concentrations ($< 2 \times 10^{-4}$ mol·dm^{-3})] in terms of eqn. 9. The appropriate parameters have been determined using non-linear regression analysis using initial estimates based on bulk-phase liquid–liquid distribution coefficients[10,36], and are given in Table II. During the analysis the pairing ion binding constant, K_2, was fixed at 2,750 mol^{-1}·dm^3 based on initial parametization of the *para*-chlorobenzoic acid data. Fig. 4 shows good linear relationships exist between log B (eqn. 9) and τ (Table I) with the *ortho* data points displaced from the *meta* and *para* values, which presumably reflects steric and intramolecular hydrogen bonding factors affecting ion-pair formation, as indicated by their lower K_1 values (Table II). Since τ has been shown (eqns. 3–6) to be correlated with substituent hydrophobicity, it follows that the alkyl benzyldimethylammonium chlorides act as pairing ions in a similar manner to that discussed above for alkylsulphates and sulphonates. Thus, the function B can now be analysed to obtain K_1 and K_3. The ion-pair chromatographic distribution constant, K_3, may be described in group contribution terms by

$$\Delta(\log K_3) = \log(K_{3_j}/K_{3_t}) \qquad (10)$$

Group chromatographic ion-pair distribution constants, $\Delta \log K_3$, and liquid–liquid distribution constants, π (Table II), can be related as shown by Fig. 5. Here the π values for benzenoids recently compiled by Norrington *et al.*[19] have been used to describe functional group bulk phase distributive properties, primarily because of the more complete data set available. Eqns. 11–14 are the most appropriate regression equations describing the relationship, *i.e.*

TABLE II

ION-PAIR FORMATION CONSTANTS, K_1, DISTRIBUTION CONSTANTS, K_3, AND π VALUES FOR SUBSTITUTED BENZOIC ACIDS

Chromatographic details: stationary phase Spherisorb ODS; mobile phase methanol–water (1:1), terdecylbenzyldimethylammonium chloride ($0.93–7.48 \cdot 10^{-4}$ mol·dm^{-3}), KH_2PO_4 ($4.76 \cdot 10^{-3}$ mol·dm^{-3}), Na_2HPO_4 ($2.02 \cdot 10^{-3}$ mol·dm^{-3}); pH 7.5; 30°C; flow-rate 2.0 ml·min^{-1}.
$\kappa^{max.}$ = maximum values derived for κ in this phase system.

Function	K_1 ($mol^{-1} \cdot dm^3$)	*Standard error*	K_3 ($mol^{-1} \cdot dm^3$)	*Standard error*	$\kappa^{max.}$	π *	π **
2-OH	856	+116	27.1	+3.6	4.6	§	−0.41
3-OH	2445	+306	4.7	+0.6	1.3	−0.38	−0.50
4-OH	3200	+504	4.1	+0.6	0.9	−0.30	−0.61
2-NH_2	762	+ 89.4	15.2	+2.1	2.1	§	−1.40
3-NH_2	1488	+153	3.8	+0.7	0.8	§	−1.29
4-NH_2	1952	+206	2.6	+0.6	0.8	§	−1.30
2-NO_2	579	+ 64.8	16.4	+1.9	1.9	§	§
3-NO_2	3087	+392	11.3	+1.5	3.8	−0.05	0.11
4-NO_2	3561	+413	11.3	+1.7	3.5	0.02	0.22
2-Cl	346	+406	35.7	+4.7	2.7	§	0.76
3-Cl	2511	+324	26.2	+4.3	7.8	0.83	0.77
4-Cl	2781	+317	26.2	+4.2	7.5	0.87	0.73
2-CH_3	608	+ 72.5	24.1	+3.3	3.0	§	0.84
3-CH_3	2570	+317	14.6	+2.0	4.5	0.52	0.52
4-CH_3	2916	+402	14.0	+1.7	4.2	0.42	0.60
H	1569	+199	11.1	+1.6	2.6	0	0

* Benzoic acids taken from refs. 13, 18 and 37.
** Benzenoid substituents, from the compilation by Norrington *et al.*[19].
§ No literature value.

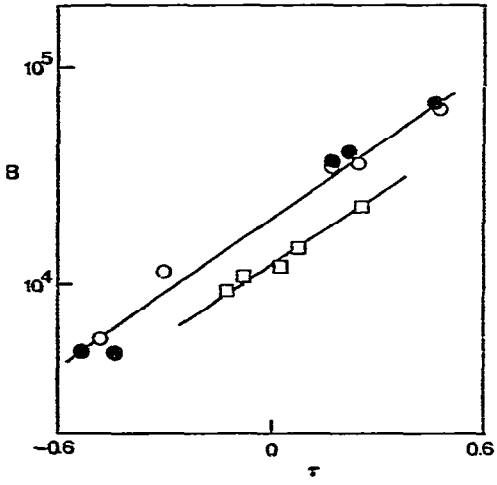


Fig. 4. Log-linear plots of B *versus* τ for benzoic acids, where B has been derived by non-linear regression of the data given in Fig. 2 according to eqn. 9 and has units of $(mol \cdot dm^{-3})^{-2}$. Key as for Fig. 2.

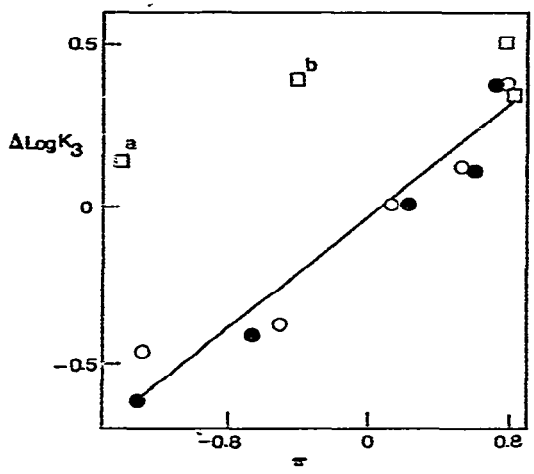


Fig. 5. Relationship between chromatographic group ion-pair distribution values, $\Delta \log K_3$, and liquid–liquid group distribution values, π,[19] for benzoic acid substituents. Plot is the regression line (eqn. 11) for the 3- and 4-substituted acids; (a) and (b) refer to 2-amino and 2-hydroxy, respectively. Key as for Fig. 2.

meta and *para* benzoic acids

$$\Delta \log K_3 = 0.43\pi - 0.06 \qquad n = 10 \qquad r = 0.964 \qquad (11)$$

$$\Delta \log K_3 = 0.58\pi - 0.12 \qquad n = 8 \qquad r = 0.948 \qquad (12)$$

all substituted benzoic acids

$$\Delta \log K_3 = 0.32\pi + 0.05 \qquad n = 14 \qquad r = 0.726 \qquad (13)$$

$$\Delta \log K_3 = 0.30\pi + 0.040\sigma + 0.04 \qquad n = 14 \qquad R = 0.709 \qquad (14)$$

σ is the Hammett electronic term[14], R the multiple correlation coefficient, π values in eqn. 12 are for substituted benzoic acids taken from the compilation of Davis *et al.*[38], and for eqns. 11, 13 and 14 from ref. 19. Eqn. 14 shows that the electronic term does not improve the relationship given in eqn. 13 for all substituted benzoic acids indicating that both electronic and steric effects can perturb the general relationship, and it is interesting to note that in Fig. 5 datum points denoted as (a) and (b) are values for the *o*-amino and *o*-hydroxy substituents, respectively.

K_1, the ion-pair formation constant, does not correlate with either π or σ, the significance of which will be discussed in the next section.

Pairing ion chain length

Since it is demonstrated that the ion-pair distribution constant is related to group τ (or π) values which are pairing ion concentration independent, and assuming that it is the total formed ion-pair which transfers to the stationary phase, it follows that an increase in pairing ion hydrophobicity although causing an increase in solute capacity ratio should not affect solute functional group selectivity. To test this hypothesis the retention behaviour of a series of substituted benzoic acids has been examined using various alkylbenzyldimethylammonium chlorides of different alkyl chain length (C_8–C_{16}) as pairing ions. Table III shows the hypothesis to be correct, *i.e.* that group selectivity is invariant with pairing ion hydrophobicity, although

TABLE III

CAPACITY RATIOS AND GROUP CONTRIBUTION VALUES DETERMINED FOR SOME SUBSTITUTED BENZOIC ACIDS, AND SODIUM CROMOGLYCATE (SCG), AND THEIR RELATIONSHIP (EQN. 15), TO THE NUMBER OF CARBON ATOMS OF THE PAIRING ION ALKYL CHAIN

Chromatographic details: stationary phase Spherisorb ODS; mobile phase methanol–water (1:1), alkylbenzyldimethylammonium chloride ($5 \cdot 10^{-4}$ mol·dm^{-3}); pH 7.5; 30°C; flow-rate 2.0 ml·min^{-1}.

Function	*Pairing ion alkyl chain length*										*Regression coefficients (eqn. 15)*		
	8		*10*		*12*		*14*		*16*				
	κ	τ	κ	τ	κ	τ	κ	τ	κ	τ	a_j	b_j	r
3-NO_2	0.60	0.18	1.00	0.20	1.82	0 16	3.09	0.17	5.89	0.24	0.12	−1.22	0.999
3-CH_3	0.71	0.25	1.10	0.24	1.95	0.19	3.39	0.21	6.17	0.26	0.12	−1.12	0.999
3-Cl	1.38	0.54	1.95	0.49	3.24	0.41	5.01	0.40	10.7	0.50	0.11	−0.78	0.990
H	0.40	—	0.63	—	1.26	—	2.09	—	3.38	—	0.12	−1.36	0.998
SCG	0.51		1.23		4.47		10.7				0.23	−2.12	0.997

retention is not. The relationship between pairing ion-alkyl chain length, n, and solute capacity ratio is given by eqn. 15:

$$\log \kappa = a_j n + b_j \tag{15}$$

where a and b are the slope and intercept coefficients for solute j. The data has been analysed according to eqn. 15 by linear regression and it is shown from Table III that the slope coefficients for all the benzoic acids can be given by 0.12 ± 0.03 standard deviations. This is the contribution each pairing ion methylene unit has towards changing solute capacity ratio under the conditions stated. It has been demonstrated previously[10] that the pairing ion methylene group contribution is dependent upon the charge of the solute reflecting the stoichiometry of the ion-pair interaction. Table III gives the regression coefficients according to eqn. 15 for the dianionic carboxylic salt sodium cromoglycate[10], and shows that the pairing ion methylene group contribution is twice that for the benzoic acid solute under the same conditions, indicating a 2:1 interaction occurring. When one considers both the stereochemistry involved and the fact that under these conditions only *ca.* 1–2% of the stationary phase surface is covered with adsorbed surfactant[10], this can only be rationalised in terms of an ion pairing in the mobile phase.

The effect of altering both pairing ion chain length and concentration has been examined using 3-nitrobenzoic acid (Fig. 6) and sodium cromoglycate (Fig. 6, ref. 10) as model solutes. Over the experimentally accessible pairing ion concentration range (due to alkylbenzyldimethylammonium chloride solubility), and using methanol–water (40:60) as the mobile phase, a hyperbolic relationship between κ and $[X]$ can be described. Data have been analysed for K_1 and K_3 using eqn. 9 transformed into the form of a hyperbolic relationship[8], *i.e.*

$$\kappa = (\kappa' + K_1 K_3 [X])\,(1 + K_1 [X])^{-1} \tag{16}$$

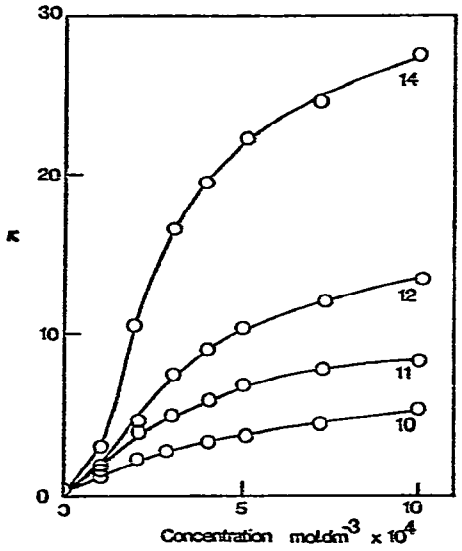
The appropriate ion-pair formation, K_1, and distribution, K_3, constants are given in Table IV. Fig. 7 gives plots of K_3 *versus* pairing ion chain length, n, for the mono-

TABLE IV

ION-PAIR FORMATION CONSTANTS, K_1, AND DISTRIBUTION CONSTANT, K_3, FOR 3-NITROBENZOIC ACID AND SODIUM CROMOGLYCATE USING PAIRING IONS OF DIFFERING ALKYL CHAIN LENGTH (SEE FIG. 8)

Chromatographic details: 3-nitro-benzoic acid, stationary phase: Hypersil ODS; mobile phase methanol–water (4:6), alkylbenzyldimethylammonium chloride ($0-1 \cdot 10^{-3}$ mol·dm^{-3}); pH 7.5, ($2.5 \cdot 10^{-2}$ mol·dm^{-3} KH_2PO_4); 30°C; flow-rate 1.5 ml·min^{-1}; sodium cromoglycate, stationary phase: Spherisorb ODS; mobile phase methanol–water (1:1), alkylbenzyldimethylammonium chloride ($0-6 \cdot 10^{-4}$ mol·dm^{-3}); pH 7.4; 31°C; flow-rate 1.5 ml·min^{-1}.

Solute	*Pairing ion alkyl chain length*									
	10		*11*		*12*		*13*		*14*	
	K_1 + *S.E.* (*mol*$^{-1}$·*dm*^{3})	K_3 + *S.E.* (*mol*$^{-1}$·*dm*^{3})	K_1 + *S.E.* (*mol*$^{-1}$·*dm*^{3})	K_3 + *S.E.* (*mol*$^{-1}$·*dm*^{3})	K_1 + *S.E.* (*mol*$^{-1}$·*dm*^{3})	K_3 + *S.E.* (*mol*$^{-1}$·*dm*^{3})	K_1 + *S.E.* (*mol*$^{-1}$·*dm*^{3})	K_3 + *S.E.* (*mol*$^{-1}$·*dm*^{3})	K_1 + *S.E.* (*mol*$^{-1}$·*dm*^{3})	K_3 + *S.E.* (*mol*$^{-1}$·*dm*^{3})
3-Nitrobenzoic acid	3244 + 1084	6.6 + 0.8	3746 + 407	10.8 + 0.4	3757 + 548	16.4 + 0.9			3654 + 352	35.7 + 1.3
Sodium cromoglycate	781 + 86	29.1 + 1.3	822 + 92	64.7 + 7.3	739 + 104	209 + 36.1	904 + 121	283 + 44.5		



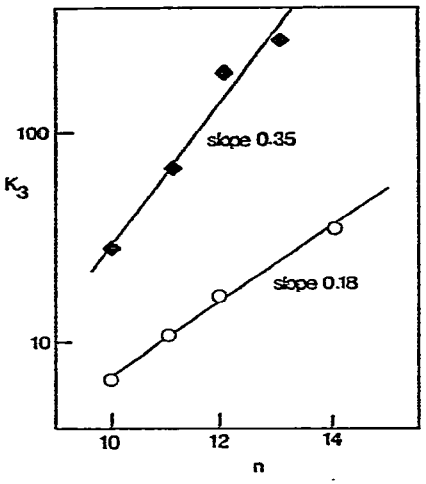


Fig. 6. Effect of pairing ion chain-length and concentration on the capacity ratio of 3-nitrobenzoic acid. Chromatographic conditions: stationary phase ODS Hypersil; mobile phase methanol–water (4:6), alkylbenzyldimethylammonium chlorides (homologue number given next to each data line), K_2HPO_4 ($2.5 \cdot 10^{-2}$ mol·dm^{-3}), pH 7.5, 30°C.

Fig. 7. Ion-pair chromatographic distribution values, K_3, *versus* pairing ion alkyl chain length n plotted on log-linear coordinates, where K_3 has units of mol^{-1}·dm^3. Closed points refer to the dianion sodium cromoglycate[10]. Chromatographic conditions: Spherisorb ODS; methanol–water (1:1). Open points refer to 3-nitrobenzoic acid, chromatographic conditions as for Fig. 6. Slope coefficients are given next to each data line.

and dianionic solutes. It is seen that the slope coefficient for sodium cromoglycate is approximately twice that for 3-nitrobenzoic acid. (Although the slightly different methanol compositions used to obtain the K_3 values would affect the absolute values of K_3 the slope coefficients should be unaltered.) The distribution coefficients, K_D, between water and chloroform for alkylbenzyldimethylammonium–cromoglycate (2:1) ion association species have recently been determined by us[36], and eqn. 17 shows that these ion-pair bulk phase liquid–liquid distribution constants are linearly related to the chromatographic distribution constants, *viz.*

$$K_3 = 7.35K_D + 23.5 \qquad n = 3 \qquad r = 0.999 \tag{17}$$

The excellent agreement between the two and the slope coefficients of the plots given in Fig. 7 further reinforce the arguments based on the meaning of the slope coefficients of eqn. 15 (Table III) that ion-pairing in the mobile phase followed by distribution to the stationary phase is the dominant retention mechanism. The assumption made in generating K_1 and K_3 from the data given in Fig. 6 concerning omitting low pairing ion concentration is seen to be justified by examination of Fig. 8 which is a normalized plot to test for the quality of the data fit of eqn. 16 in terms of K_1, K_3 and $[X]$ describing a hyperbolic relationship passing through the origin.

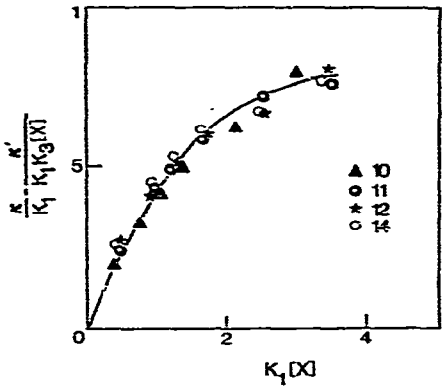


Fig. 8. Plot of normalised capacity ratio (ordinate) *versus* normalised pairing ion concentration (abscissa) for the data given by Fig. 6, according to eqns. 9 and 16. Theoretically the relationship describes a rectangular hyperbola passing through the origin and this is the case for the above illustrating the good quality of fit of data. (Numbers refer to pairing ion chain length).

It is extremely interesting that the ion-pair formation constants (Tables II and IV) are largely independent of solute hydrophobicity and within limits, appear to be dependent upon the magnitude of the electrical charge. Ion-pairing in water between large organic ions can be shown[36,39,40] to be reinforced by hydrophobic interactions, such as envisaged by Diamond[41], so it must be concluded that in the present systems the high percentage of organic modifier in the mobile phase tends to blur this dependency on water structure effects.

Organic modifier

Ion pairs formed in the systems under study although large (mol. wt. > 400) and hydrophobic still retain some polarity[42], and since liquid–liquid distribution of ion pairs is described better in terms of specific solvate formation rather than regular solution behaviour[43], it is seen that alteration in mobile phase composition should cause a complex alteration in both retention and selectivity. Using methanol as the organic modifier (34–60%), ODS Hypersil as the stationary phase, with a fixed tetradecylbenzyldimethylammonium pairing ion concentration ($5 \cdot 10^{-4}$ mol·dm^{-3}), and at pH 7.5 (K_2HPO_4 buffer, 0.025 mol·dm^{-3}) the retention behaviour of a series of ten substituted benzoic acids has been determined (Table V). Fig. 9 shows that although there is generally a linear relationship between τ and methanol concentration over the studied range, for the strongly hydrogen bonding hydroxy substituents non-linearity is exhibited.

Group contribution data has been analysed using linear free-energy relationships with respect to methanol percentage composition (eqn. 18), and at each methanol composition with respect to π (eqn. 19). The forms of these equations are given below and the regression coefficients are given in Table V together with appropriate statistical information, *i.e.*

$$\tau = c[\text{organic modifier}] + d \tag{18}$$

$$\tau = e\pi + f \tag{19}$$

TABLE V

CAPACITY RATIOS AND GROUP CONTRIBUTION VALUES FOR SOME SUBSTITUTED BENZOIC ACIDS WITH CHANGE IN MOBILE PHASE METHANOL PERCENT COMPOSITION; REGRESSION COEFFICIENTS FOR GROUP DATA ANALYSIS ACCORDING TO EQNS. 18 AND 19

Chromatographic details: stationary phase Hypersil ODS; mobile phases methanol–water, tetradecylbenzyldimethylammonium chloride ($5 \cdot 10^{-4}$ mol·dm^{-3}), K_2HPO_4 ($2 \cdot 10^{-2}$ mol·dm^{-3}); pH 7.5; 30°C

Function	*Mobile phase percent methanol composition (by volume)*								*Regression coefficients according to eqn. 18*		
	60		*50*		*40*		*34*				
	κ	τ	κ	τ	κ	τ	κ	τ	*c*	*d*	*r*
3-OH	0.69	−0.26	1.91	−0.30	5.50	−0.24	15.1	−0.18	−0.003	−0.10	0.72
4-OH	0.51	−0.38	1.10	−0.54	2.82	−0.53	6.76	−0.54	0.006	−0.75	0.81
3-NH_2	0.45	−0.45	1.00	−0.58	2.34	−0.61	4.57	−0.71	0.010	−1.01	0.97
4-NH_2	0.32	−0.59	0.68	−0.75	1.38	−0.84	2.24	−1.01	0.015	−1.49	0.98
3-NO_2	1.70	0.13	6.31	0.22	18.6	0.29	50.1	0.33	−0.008	0.59	0.99
4-NO_2	1.66	0.12	5.62	0.17	17.0	0.25	44.7	0.29	−0.007	0.51	0.99
3-Cl	3.16	0.41	13.5	0.55	43.7	0.66	125	0.74	−0.013	1.17	0.99
4-Cl	2.95	0.38	12.9	0.53	42.7	0.65	125	0.74	−0.014	1.20	0.99
3-CH_3	2.19	0.24	8.13	0.33	22.9	0.38	64.6	0.44	−0.007	0.69	0.99
4-CH_3	2.14	0.23	7.59	0.30	21.4	0.35	64.6	0.39	−0.006	0.59	0.99
H	1.26	—	3.80	—	9.54	—	23.4	—			

*Regression coefficients according to eqn. 19**

e	0.45	0.60	0.68	0.76
f	0.02	0.04	0.09	0.11
r	0.983	0.976	0.977	0.978

* Using π values from ref. 19.

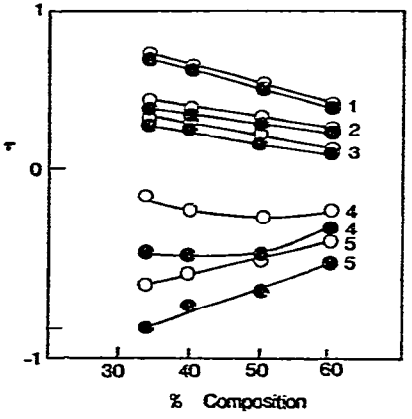


Fig. 9. Relationship between τ and percent composition of methanol for benzoic acids. Chromatographic conditions: stationary phase ODS Hypersil; mobile phase methanol–water, tetradecylbenzyldimethylammonium chloride ($5 \cdot 10^{-4}$ mol·dm^{-3}), K_2HPO_4 ($2.5 \cdot 10^{-2}$ mol·dm^{-3}); pH 7.5; 30°C. Key as for Fig. 2.

The perturbation of the linear relationships given by eqn. 18 by hydroxyl groups is minimized using eqn. 19, as shown by the found correlation coefficients (Table V). The good fit of the data by eqn. 19 suggest that published functional group hydrophobicity parameters, (*e.g.* ref. 43), can be used to indicate phase selectivity in ion-pair chromatography between solutes differing by one functional group, (as is often so in drug stability and metabolism studies). Alternatively, RP-HPLC using surface active pairing ions may be used to generate a data bank of hydrophobicity parameters for use in, for example, drug design models[26].

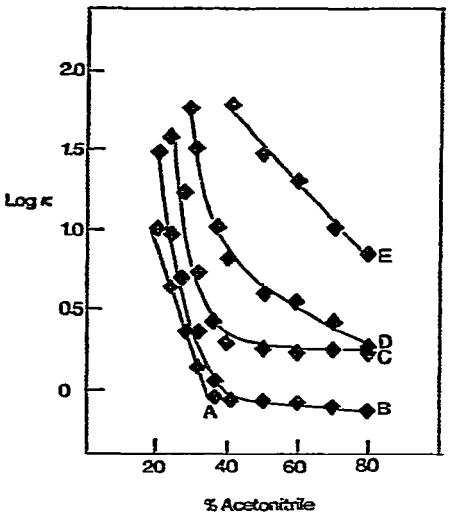
Although the use of extra-thermodynamic linear free-energy relationships can provide a semi-empirical approach for defining retention behaviour in ion-pair systems, it reveals little of the reasons why group selectivity changes with alteration in organic modifier type or concentration. To attempt to study these reasons, the capacity ratios of a series of 1,3,5-*s*-triazines have been determined (Table VI) using methanol (30–96%) or acetonitrile (20–96%) as the organic modifier. The triazine series was chosen because of (a) their known physico-chemical properties[20], (b) availability of very polar (*e.g.* SO_2NH_2) and hydrophobic (*e.g.* O-*n*-C_9H_{19}) analogues, and (c) their retention behaviour generally permitted a wide range of organic modifier concentrations to be studied. Table VI and Fig. 10 show that over a large organic modifier concentration the relationship with κ is non-linear. This is particularly true for acetonitrile. Fig. 10 shows that log κ falls rapidly between 20 and 40% acetonitrile followed by a plateau between 40 and 70% acetonitrile, followed by another fall at higher concentrations. These effects are less pronounced for very hydrophobic analogues. A comparison of the effects of acetonitrile and methanol on the retention of the parent triazine molecule is given by Fig. 11a, which shows that below 70% organic modifier, retention is greater with methanol–water mobile phases compared to acetonitrile–water phases. The opposite is found above 70% organic modifier. Similar effects are generally found for functional group values as shown by Fig. 12.

An indication as to the dominant feature controlling selectivity and retention in ion-pair RP-HPLC systems using water–methanol and water–acetonitrile mobile phases, can be gathered from Fig. 11b which shows the relationship between surface tension, γ, and percentage organic modifier. The marked similarity between the two curves shown and those given in Fig. 11a is augmented by the crossover in both curves being at *ca.* 70% organic modifier.

Application[25,44] of solvophobic theory[24] to RP-HPLC of unionised solutes, and the experimentally observed chromatographic behaviour of weak acids with water–methanol and water–acetonitrile mobile phases, shows that the relationship between retention and eluent surface tension (for these eluents) may be given by

$$\ln \kappa = g + \frac{N\Delta A + 4.836N^{1/3}(k^c - 1)V^{2/3}}{RT}\gamma \qquad (20)$$

where g is a constant; N, R, T and V are Avogadro's number, the gas constant, absolute temperature and average molar volume of the mobile phase, respectively; ΔA is the relative surface area of the solute molecule in contact with the stationary phase, and can be indicated by molecular surface area[45]; and k^c may be defined[25] as the ratio of the energy required to create a cavity for a solvent molecule to the energy required to extend the planar surface of the solvent by the surface area of the added



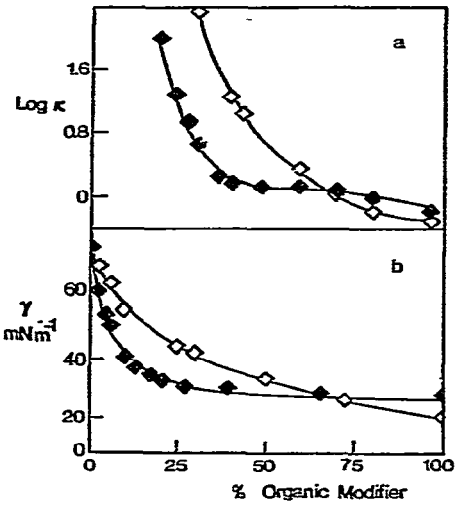


Fig. 10. Relationship between log κ and percent acetonitrile mobile phase composition for some substituted 1,3,5-*s*-triazines (Table VI). A–E refer to the 3-SO_2NH_2, 4-$NHCOCH_3$, 3-$N(CH_3)_2$, 3-$C(CH_3)_3$ and 3-O-*n*-C_9H_{19} substituted triazines, respectively.

Fig. 11. Relationship between percent organic modifier composition and (a) log capacity ratios for unsubstituted 1,3,5-*s*-triazine, and (b) mobile phase surface tensions (γ). Open and closed points refer to methanol and acetonitrile organic modifier, respectively.

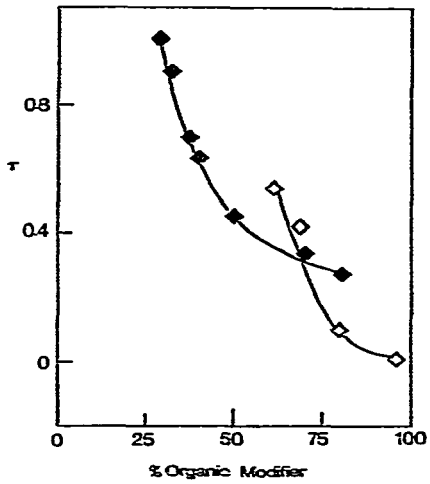


Fig. 12. Relationship between 3-$C(CH_3)_3$ τ values (triazine series) and percent organic modifier composition. Key as for Fig. 11.

solute molecule. By application of the group contribution approach (eqn. 1) to the relationship given by eqn. 20, we may obtain

$$\tau_{jl} = \frac{N\gamma(\Delta A_j - \Delta A_l)}{2.3\,RT} \qquad (21)$$

TABLE VI

CAPACITY RATIOS FOR SUBSTITUTED TRIAZINES AT VARYING METHANOL-WATER AND ACETONITRILE-WATER MOBILE PHASE COMPOSITIONS

Chromatographic details: stationary phase Hypersil ODS; mobile phase organic modifier-water, sodium dodecylsulphate ($5 \cdot 10^{-4}$ mol·dm^{-3}), 0.1% H_2SO_4; pH 2.2; 30°C.

Function	*log k*											*log k*						
	Mobile phase percent acetonitrile composition (by volume)											*Mobile phase percent methanol composition (by volume)*						
	20	*24*	*28*	*32*	*36*	*40*	*50*	*60*	*70*	*80*	*96*	*30*	*40*	*44*	*60*	*70*	*80*	*96*
3-OH	1.44	0.96	0.65	0.35	0.08													
4-OH	1.42	0.88	0.58	0.30	0.07								0.87	0.63	−0.04	−0.26		
4-$NHCOCH_3$	1.49	0.97	0.68	0.36	0.04	−0.04	−0.08	−0.09	−0.10	−0.13		1.70	1.04	0.75	0.08	−0.14	−0.26	−0.32
3-$N(CH_3)_2$		1.60	1.24	0.71	0.40	0.24	0.26	0.27	0.28	0.24								
3-SO_2CH_3	1.25	0.83	0.58	0.21	0.08							1.38	0.77	0.55	−0.04			
4-SO_2NH_2	1.03	0.63	0.39	0.13	−0.06							0.94	0.48	0.25	−0.25	−0.50		
3-CN	1.50	1.05	0.79	0.49	0.20													
3-$C(CH_3)_3$			1.98	1.51	1.01	0.82	0.59	0.56	0.41	0.23					0.87	0.41	−0.07	−0.32
3-O-*n*-C_6H_{13}						1.38	1.02	1.06	0.82	0.58					1.33	0.88	0.44	−0.12
3-O-*n*-C_9H_{19}						1.80	1.48	1.30	1.02	0.84							0.76	−0.04
3-SCH_3		1.76	1.40	0.99	0.60	0.43	0.32	0.29	0.18	0.04					0.44	0.07	−0.10	−0.32
3-CF_3		1.81	1.46	1.07	0.66	0.44	0.33	0.25	0.14	−0.03								
3-Br		1.71	1.35	0.99	0.59								1.60	1.34	0.46	0.12	−0.07	−0.26
3-$(CH_2)_4$-phenyl						1.37	1.02	0.88	0.60	0.31								
3-$(CH_2)_4$-phenyl-4′-OCH_3						1.42	1.09	1.00	0.71	0.39								
H	1.81	1.27	0.93	0.61	0.31	0.18	0.14	0.15	0.10	−0.05	−0.18	2.00	1.25	1.00	0.33	−0.02	−0.16	−0.30

For methanol–water or acetonitrile–water mixtures the term g in eqn. 20 is largely related to the van der Waals component of the free-energy of interaction of the solute with the solvent, which can be given by $\Delta G_{vwd}/RT$ (eqn. 46, ref. 25). This will change with change in organic modifier composition, hence eqn. 21 needs to be modified to include this function so that in group terms:

$$\tau_{jl} = \frac{\Delta(\Delta G_{vdw})_{jl} + \gamma N(\Delta A_j - \Delta A_l)}{2.3\,RT} \tag{22}$$

where the term $(\Delta A_j - \Delta A_l)$ is a group constant describing the effect of a substituent in altering the surface contact area of the solute with the stationary support.

The data given in Table VI have been analysed using eqn. 22 and presented graphically by Figs. 13a and 13b. Eqn. 22 and Fig. 11b show that in ion-pair RP-HPLC using surface-active pairing ions, there should be a linear relationship between τ and γ, and that for water–methanol and water–acetonitrile eluents the relationship should converge at $\tau = 0$ and $\gamma \approx 21$ and 29 $mN \cdot m^{-1}$, respectively, with intercepts equal to $\Delta(\Delta G_{vdw})_{jl}/2.3RT$. Fig. 13a and b show both the premise and the prediction to be correct. These results imply that both ion-pair formation and distribution to the stationary phase are affected similarly by changes in surface tension. Literature values[46,47] of surface tensions have been used in this study, and hence the linear relationships between τ and γ show that the effect of buffer salt and added surfactant on γ is constant over the organic modifier concentration range examined. However, there will be alterations in γ due to buffer salt, surfactant and solute; this could explain the small displacements of the τ *versus* γ plots seen for some substituents (Fig. 13).

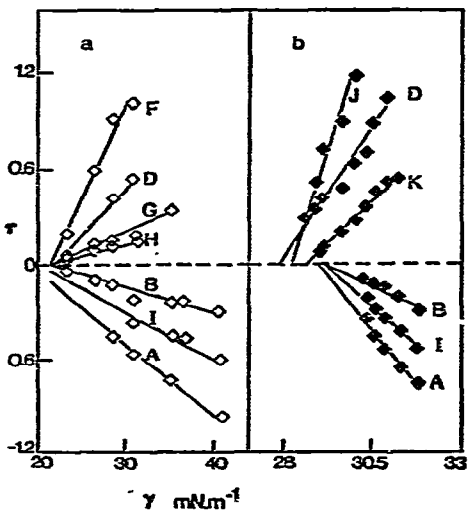


Fig. 13. Relationships between τ and mobile phase surface tension (γ) using (a) methanol and (b) acetonitrile as the organic modifier. Chromatographic details given in Table VI. Substituent key: A, B, D as for Fig. 10, F–K are 3-O-n-C_6H_{13}, 3-Br, 3-SCH_3, 3-SO_2CH_3, 3-$(CH_2)_4$-phenyl, and 3-CF_3, respectively. Coincidental plots have been omitted for clarity purposes.

These results add testimony to the usefulness of using solvophobic theory to rationalise solute retention in RP-HPLC[8,25,44], and from a pragmatic viewpoint show that group selectivity in ion-pair systems using surface active pairing-ions could be estimated[5,23] for different mobile phases using surface tension values, although for multi-component eluents[31,35] the lack of literature γ values would make this tedious.

Operationally, column efficiency in ion-pair RP-HPLC is affected in a complex way by altering mobile phase organic modifier composition and concentration. There is an apparent relationship between reduced plate height at various percent organic modifier concentrations and eluent viscosity[48], and we are currently investigating this phenomena more thoroughly.

Ionic strength

A priori the results found in the previous section suggest that any increase in surface tension would increase both retention and group selectivity. An increase in ionic strength causes the surface tension of alcohol–water mixtures to rise indicating a rise in κ with increasing ionic strength. Results in this present study and those given elsewhere[5,8,23,31,34] are to the contrary for ion-pair systems. Fig. 14a and b show the relationships between capacity ratio and ionic strength for the benzoic acid solute series using either potassium nitrate or dipotassium hydrogen orthophosphate to adjust the ionic strength.

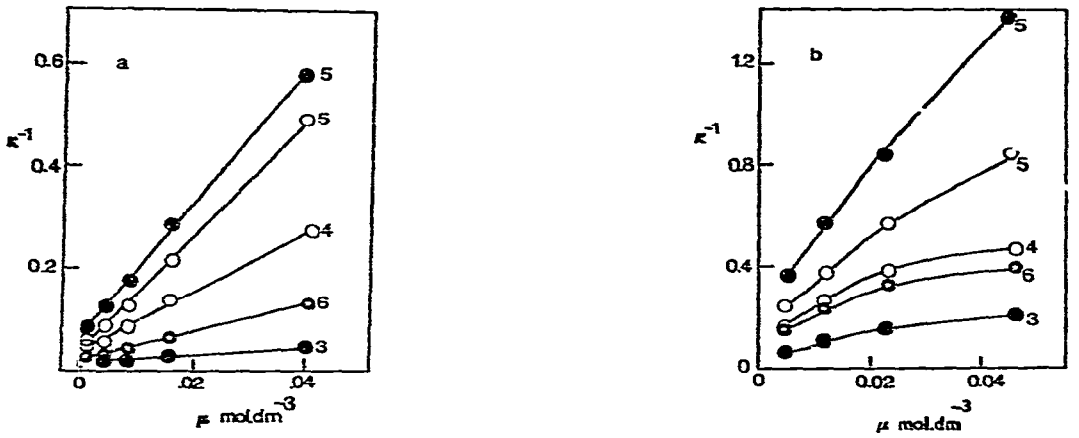
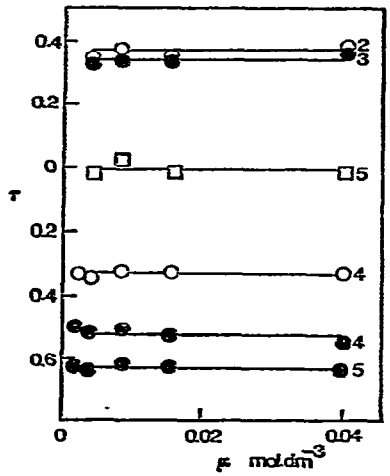


Fig. 14. Relationships between reciprocal capacity ratios for benzoic acids and ionic strength using (a) KNO_3 and (b) K_2HPO_4 as salt. Chromatographic conditions: stationary phase Spherisorb ODS; mobile phase acetonitrile–water (2:8), terdecylbenzyldimethylammonium chloride ($3.5 \cdot 10^{-4}$ mol·dm^{-3}), salt; pH 7.5 (adjusted by dropwise addition of either NaOH or HCl; 30°C. Key as for Fig. 2.

The results can be explained empirically by recourse to the presumed retention model. We have shown[49] that transfer of cromoglycate ions as ion-pairs with alkylbenzyldimethylammonium ions from water to chloroform is reduced markedly on the addition of small amounts of NaCl (0.01–0.06 mol·dm^{-3}), and that this is related to a reduction in the association constant between the two ions[50], due to shielding of the ions' charge centers[41]. If this is the case for the systems under study then τ should

be independent of ionic strength; Fig. 15 shows this to be the case using KNO_3 as the added salt at the concentrations of salt generally found in HPLC analyses. (A similar insensitivity of τ for ionic strength can be determined from other reported studies, *e.g.* ref. 5). The addition of salt can alter ion-pair equilibria in other ways[4]. Added salt can compete with the pairing ion in forming ion pairs with the solute ion, or conversely it can form ion pairs with the pairing ion so reducing the thermodynamic activity of the latter, and these effects are probably reflected by the non-linearity of the κ^{-1} *versus* μ plot found using high ionic strengths with KH_2PO_4 as added salt (Fig. 14b).



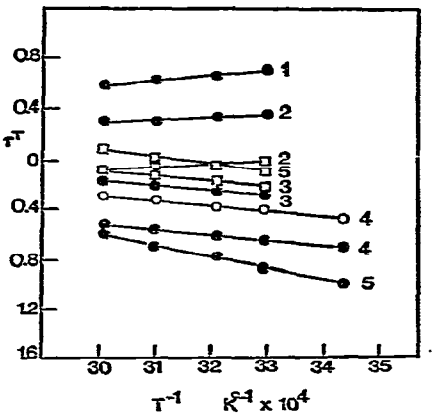


Fig. 15. Effect of ionic strength (μ) of mobile phase on τ for benzoic acid substituents. Conditions and key as for Fig. 14a.

Fig. 16. Van 't Hoff plots for benzoic acid substituents showing τ *versus* reciprocal temperature, T^{-1} (°K). Chromatographic conditions: stationary phase Spherisorb ODS; mobile phase acetonitrile–water (2:8), terdecylbenzyldimethylammonium chloride ($5 \cdot 10^{-4}$ mol·dm^{-3}), K_2HPO_4 ($2.5 \cdot 10^{-2}$ mol·dm^{-3}), pH 7.5. Key as for Fig. 2. Coincidental plots have been omitted for clarity purposes.

Temperature

The effect of temperature on retention in ion-pair HPLC is reported generally in qualitative terms (*e.g.* ref. 29) and there have been only a few instances[5,51,52] where detailed studies have been made, although it is often shown that an increase in temperature will improve peak shape and shorten retention times.

In LC solute distribution from a mobile to a stationary phase is exothermic, that is, an increase in temperature causes a fall in retention. This is a particularly intriguing observation for RP-LC since this is presumed to be effected by the same physico-chemical equilibria which are responsible for the hydrophobic process, (a process regarded[53] as being entropically driven and endothermic, that is, a positive ΔS and ΔH). Over the normally accessible temperature range retention can be related[25,53] to temperature by a modified Van't Hoff equation, *viz*:

$$\ln \kappa = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \varphi \qquad (23)$$

where ΔH^0 and ΔS^0 are the standard enthalpy and entropy change for solute transfer to the stationary phase. The last term in eqn. 23 is difficult to determine for brush-type reversed-phase packings, however, using a group contribution we obtain

$$\tau = -\frac{\Delta(\Delta H^0)}{2.3\,RT} + \frac{\Delta(\Delta S^0)}{2.3\,R} \tag{24}$$

Fig. 16 shows Van't Hoff plots according to eqn. 24 for functional groups derived from benzoic, phenylacetic and cinnamic acid solute series. Although solute enthalpy terms are all negative for these series (Table VII) the groups contribute exothermically or endothermically to the transfer. Enthalpy values for other ion-pair HPLC systems can be calculated or obtained from the literature and range from *ca.* −16 to *ca.* −35 kJ·mol^{-1} for amino acid solutes[5] using alkylsulphate as pairing ion, to *ca.* −60 to −90 kJ·mol^{-1} for anionic dyes[52] using small alkylammonium pairing ions. These values, and our own are generally higher than those reported for comparable non-ion-pair HPLC systems (*e.g.* refs. 54–59).

TABLE VII

FUNCTIONAL GROUP ENTHALPIC CONTRIBUTIONS AND τ VALUES AT THE HARMONIC MEAN TEMPERATURE DETERMINED FOR BENZOIC ACIDS

Chromatographic details: stationary phase Spherisorb ODS; mobile phase acetonitrile–water (1:4), terdecylbenzyldimethylammonium chloride ($5 \cdot 10^{-4}$ mol·dm^{-3}); K_2HPO_4 ($2.5 \cdot 10^{-2}$ mol·dm^{-3}); pH 7.5; flow-rate 1.5 ml·min^{-1}; temperature range 18.8–60.5°C.

Function	$\tau^{40°}$**	$\Delta(\Delta H°)$ (kJ·mol^{-1})
3-OH	−0.38	4.8
4-OH	−0.61	7.2
2-NH_2	−0.04	−2.8
4-NH_2	−0.77	14.0
2-NO_2	−0.18	7.6
4-NO_2	0.32	3.9
2-Cl	−0.05	10.0
4-Cl	0.66	−8.2
2-CH_3	−0.06	13.2
4-CH_3	0.32	−5.6
CH=CH*	0.40	−3.0

Benzoic acid ($\kappa^{40°} = 11.0$), $\Delta H° = -34.5$ kJ·mol^{-1}.

* Cinnamic acid.

** Extrapolated values from Fig. 16.

Leffler and Grunwald[11] have shown that to identify a single mechanism of interaction for a series of solutes if ΔH and ΔS are approximated as being constant then $\delta\Delta H$ should be simply proportional to $\delta\Delta S$ (where δ denotes a change caused in the thermodynamic parameter by a medium effect, or, as is the case for this present study, by a change in substituent). Others[60,61] have shown, however, that this extra-

thermodynamic[11] analysis of enthalpy–entropy data can lead to artifacts caused not by true compensation between the two but by statistical effects. Accordingly the data in this present study has been analysed by a method first applied to non-ion-pair RP-HPLC systems by others[55], such that in group contribution terms we may write:

$$\tau^T = -\frac{\Delta(\Delta H^0)}{2.3\,R}\left(\frac{1}{T}-\frac{1}{\beta}\right)-\frac{\Delta(\Delta G^0)\,\beta}{2.3\,R\beta} \qquad (25)$$

where T is the harmonic mean of the experimental temperatures studied; hence τ^T are group values obtained at this temperature (which for the present study is taken as 40°C) by linear extrapolation of the data in Fig. 16, (using harmonic mean temperature data minimizing statistical compensation behaviour[60,61]). β is a proportionality factor as defined by Leffler and Grunwald[11], and having the dimensions of absolute temperature has come to be termed the "compensation temperature" such that near temperature β the free energy of the process is largely unaffected by temperature due to changes in ΔH being compensated for by changes in ΔS.

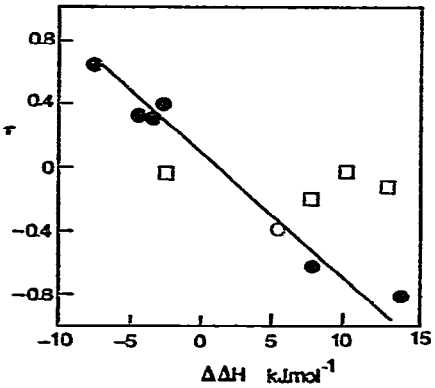


Fig. 17. Enthalpy–entropy compensation plot (τ^T-$\Delta\Delta H$ coordinates) for benzoic acid substituents derived from harmonic mean temperature values (Table VII). Regression line shown is according to eqn. 26. Chromatographic conditions and key as for Fig. 16.

Fig. 17 is the appropriate compensation plot for functional groups determined in an ion-pair RP-HPLC system. The relationship is given by eqn. 26 (where the 2-substituents have been omitted)

$$\tau^T = -0.068\Delta(\Delta H^0) + 0.043 \quad n = 7 \quad r = 0.976 \qquad (26)$$

which corresponds to a compensation temperature of *ca.* 770°K. The good fit shown is indicative of a common retention mechanism and supports the arguments given in the preceding sections. (It is useful to note that compensation behaviour has been shown[50,62] recently for complexation between large organic ions of opposite charge in water).

For neutral solutes in RP-HPLC we have demonstrated[54,58] a similar relationship to eqn. 24, *i.e.*[58]

$$\tau^T = -0.076\Delta(\Delta H^0) + 0.02 \quad n = 28 \quad r = 0.931 \qquad (27)$$

The data used to generate eqn. 27 was taken from the retention behaviour of 100 substituted alkylbenzoates determined in octyl and octadecyl RP systems using water–methanol as the mobile phase. The similarities between the regression coefficients of eqns. 26 and 27 indicate that the relationship is a general one for RP-HPLC using, at least, water–methanol and water–acetonitrile mobile phases. Although the predictive ability of those equations will be interesting to determine, it should be realised that $A(\Delta H^0)$ values are larger than those generally found in adsorption RP-HPLC, and must reflect the two-stage mechanism of retention.

Since values of τ decrease with increasing temperature, in terms of selectivity there is no advantage in raising temperature, although in common with other workers for this present study we have observed a reduction in the reduced plate height with a temperature increase by a factor of *ca.* $\times$ 3 with a 40°C increase. This can be rationalised qualitatively in terms of temperature effects on the viscosity of water–methanol mixtures[63]. Interestingly Laub and Purnell[64] have analysed the ion-pair temperature data of Kraak *et al.*[5] in terms of optimal operational selectivity regions and find that these are achieved at 15°C. An analysis of the present data in terms of the approach of Laub and Purnell, (possibly modified to being multivariate), should prove interesting.

Stationary phase material

Of concern to us during this study has been the quality and properties of the stationary phase material used, particularly with regard to the reproducibility of τ values with different stationary phases and the ethos of the predictability of the group approach. To assess the chromatographic properties of packing materials the retention and group selectivity behaviour for model solutes of five commercially available chemical bonded stationary phases have been studied. To assess the potential adsorption power of residual silanol groups of these phases, the retention behaviour of nitrobenzene has been determined using dry *n*-hexane as mobile phase[65]. Table VIII shows that silanization reduces the adsorptive power of the silica surface almost completely (with *n*-hexane the steric nature of the alkyl brush in limiting adsorption is slight since κ for nitrobenzene on Spherisorb ODS is less than on Partisil ODS-2, despite the latter having a much higher carbon loading).

Table VIII includes the appropriate group τ values and parent κ values for substituted benzoic, phenylacetic and cinnamic acids, determined using tetradecylbenzyldimethylammonium as the pairing ion, and aqueous-methanol mobile phases. The retentive power of the stationary phases is ranked as Partisil ODS-2 > Hypersil ODS > Spherisorb ODS > Spherisorb Hexyl > Partisil ODS, and can be related to the carbon loading of the support. It has been demonstrated recently[66] that for neutral solutes in RP-HPLC selectivity increases with increasing bonded-phase carbon content up to about 15%, above which it stays approximately constant. For the present study it can be shown (Fig. 18) that although τ is sensitive to percent carbon loading this is only significant for polar groups, and for solvophobic functions (positive τ) the values are relatively invariant. For the various packings τ can be correlated with π values according to eqn. 19 and derived regression coefficients and statistical information are given in Table VIII. The slope coefficient is a measure of stationary phase selectivity and depends on carbon loading. The significant positive intercept produced for the Partisil ODS data can be attributed to the effect of residual hydroxyl groups

TABLE VIII

FUNCTIONAL GROUP BEHAVIOUR FOR BENZOIC ACIDS DETERMINED USING VARIOUS ALKYLSILICA STATIONARY PHASES

Packing material	*Hypersil ODS*		*Spherisorb ODS*		*Partisil ODS-2*		*Partisil ODS*		*Spherisorb Hexyl*	
*Residual silanol groups**	*Capped*		*Part capped*		*Present*		*Present*		*Capped*	
*Carbon content (%)***	9.9		5.9		15.0		3.9			
*Nitrobenzene behaviour ($\kappa=$)***	0.19		3.1		3.6		5.80		0.56	
*Ion-pair HPLC****	κ	τ	κ	τ	κ	τ	κ	τ	κ	τ
Function										
2-OH	8.5	0.35	5.5	0.30	24.0	0.37	2.1	0.21	5.2	0.30
3-OH	1.9	−0.30	1.3	−0.34	3.1	−0.52	0.65	−0.30	1.4	−0.28
4-OH	1.1	−0.54	1.0	−0.44	2.1	−0.67	0.89	−0.17	0.89	−0.37
2-NH_2	3.1	−0.09	2.8	0.00	8.3	−0.09	1.2	−0.03	2.1	−0.10
3-NH_2	1.0	−0.58	0.87	−0.49	1.9	−0.72	0.46	−0.45	0.89	−0.37
4-NH_2	0.74	−0.71	0.54	−0.70	1.3	−0.89	0.35	−0.58	0.66	−0.60
2-NO_2	3.1	−0.09	1.7	−0.19	6.0	−0.22	1.1	−0.05	2.2	−0.08
3-NO_2	6.3	0.22	4.1	0.17	15.8	0.19	2.2	0.22	3.5	0.12
4-NO_2	5.6	0.17	3.9	0.16	15.8	0.19	2.0	0.18	3.3	0.11
2-Cl	4.5	0.07	2.3	−0.07	8.5	−0.07	1.2	−0.03	3.1	−0.02
3-Cl	13.5	0.55	8.1	0.48	36.3	0.55	4.7	0.55	7.4	0.45
4-Cl	12.9	0.53	7.9	0.47	38.0	0.57	4.7	0.55	7.2	0.44
2-CH_3	4.9	0.11	2.7	0.00	11.0	0.03	1.5	0.06	3.4	0.11
3-CH_3	8.1	0.33	4.7	0.24	21.9	0.33	2.6	0.30	5.2	0.30
4-CH_3	7.6	0.30	4.6	0.22	20.4	0.31	2.6	0.31	5.0	0.28
CH_2‡	4.0	0.02	2.8	0.00	10.0	0.01	1.5	0.07	2.8	0.02
CH=CH‡‡	8.5	0.35	5.1	0.27	21.4	0.32	2.9	0.34	2.2	0.33
H	3.8	—	2.8	—	10.2	—	1.3	—	2.6	—
Regression coefficients for eqn. 18 for 3- and 4-substituents										
e	0.60		0.52		0.69		0.50		0.46	
f	0.04		0.02		−0.01		0.10		0.04	
r	0.976		0.972		0.974		0.979		0.972	

* Manufacturer's description.

** Mobile phase, dry *n*-hexane. Capacity ratio on a straight phase silica (Partisil 10) is 6.80.

*** Mobile phase, methanol–water (1:1), tetradecylbenzyldimethylammonium chloride ($5.0 \cdot 10^{-4}$ mol.dm^{-3}), K_2HPO_4 ($2.5 \cdot 10^{-2}$ mol.dm^{-3}); pH 7.5; 30°C; flow-rate 2.0 ml·min^{-1}.

‡ Phenylacetic acid.

‡‡ Cinnamic acid.

on the τ value for the 4-hydroxyl functions. The rank order for the value of the slope coefficients is not the same as that for order of retentive power (Table VIII) in that Spherisorb Hexyl is less selective than Partisil ODS whilst at the same time being a more retentive phase. Since for the other octadecyl phase selectivity is proportional to retention this means that in ion-pair RP-HPLC the shorter C_6 bonded phase is less useful than the C_{18} bonded phase in terms of selectivity.

These findings and Hennion *et al.*'s study[66], give us confidence in suggesting

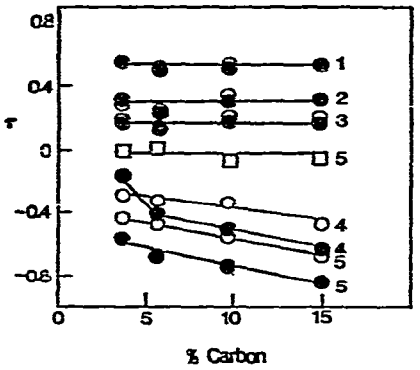


Fig. 18. Effect of octadecyl stationary phase percent carbon loading on τ (Table VIII). Key as for Fig. 2.

that τ values will be interconvertible for the more modern packing materials having surface carbon-coverage of $> 10\%$ and no residual silanol groups. The predictive relevance of this is obvious. Other workers[5,15,67] have examined retention behaviour in ion-pair RP-HPLC using packing materials of different bristle length and although it has been argued that bristle length has an effect only on retention and not on selectivity[67] these results must be examined in the light of the findings for ion-pair systems presented here and those of Hennion *et al.*[66] and Colin *et al.*[68] for non ion-pair RP-HPLC which show the effect of carbon loading on selectivity

CONCLUSIONS

The effects of environmental and constitutional variables on the retention behaviour of functional groups having widely differing character have been examined in ion-pair RP-HPLC using large surface active pairing ions.

Chromatographic group contribution values (τ) are found to be directly related to liquid–liquid distribution group parameters (π)[19], indicating (a) hydrophobic effects dominate subsequent to ion-pairing, and (b) that either π values could be used to predict τ values, or conversely, the more readily experimentally determined τ values could be used as hydrophobic parameters, for example, in drug structure activity relationship modelling[26].

It is demonstrated that the use of solvophobic theory[24] as suggested by Horváth *et al.*[25] provides a general framework for rationalizing many of the observations; in particular, retention has been characterized as due to ion-pairing in the mobile phase followed by distribution to the stationary phase, and the observed non-linearity between pairing ion concentration and capacity ratio has been analyzed for both ion-pair formation and distribution equilibria constants. The latter are demonstrated as being directly related to both π and bulk phase ion-pair liquid–liquid distribution coefficients[36]. Mobile phase effects on group contribution values have been related directly to surface tensions of these aqueous organic mixtures, as predicted by solvophobic theory considerations. For various stationary phases the effect of carbon loading on τ is found to be significant only for polar groups. All these findings are suggested as being useful for prediction of retention behaviour.

Similar to non-ion-pair chromatographic studies[54,55,58] we find that group behaviour exhibits enthalpy–entropy compensation behaviour (as determined using ΔH-ΔG coordinates), suggesting a common mechanism of retention.

The findings presented here have been reported briefly in a previous communication[69].

ACKNOWLEDGEMENTS

We are grateful to the Science Research Council and Imperial Chemical Industries for a postgraduate studentship for one of us (C.M.R.); to Dr. K. R. H. Wooldridge (May and Baker, Dagenham, Great Britain) for his interest in the section dealing with drug structure–biological activity relationships and for providing us with samples of substituted azapurines and triazines. The valued secretarial assistance of Ms. S. Sheffield is warmly acknowledged.

REFERENCES

1 G. Schill, in E. Reid (Editor), *Methodological Surveys in Biochemistry, Vol. 7, Blood Drugs and Other Analytical Challenges*, Ellis Horwood, Chichester, 1978, p. 195.
2 D. P. Wittmer, N. O. Nuessle and W. G. Haney, *Anal. Chem.*, 47 (1975) 1422.
3 J. H. Knox and J. Jurand, *J. Chromatogr.*, 125 (1976) 89.
4 E. Tomlinson, T. M. Jefferies and C. M. Riley, *J. Chromatogr.*, 159 (1978) 315.
5 J. C. Kraak, K. M. Jonker and J. F. K. Huber, *J. Chromatogr.*, 142 (1977) 671.
6 J. H. Knox and G. R. Laird, *J. Chromatogr.*, 122 (1976) 17.
7 J. L. M. Van der Venne, J. L. H. M. Hendrikx and R. S. Deelder, *J. Chromatogr.*, 167 (1978) 1.
8 C. Horváth, W. Melander, I. Molnár and P. Molnár, *Anal. Chem.*, 49 (1977) 2295.
9 C. M. Riley, E. Tomlinson, T. M. Jefferies and P. H. Redfern, *J. Chromatogr.*, 162 (1979) 153.
10 E. Tomlinson, C. M. Riley and T. M. Jefferies, *J. Chromatogr.*, 173 (1979) 89.
11 J. E. Leffler and E. Grunwald, *Rates and Equilibria of Organic Reactions*, Wiley, New York, London, 1963, pp. 128–314.
12 I. E. Bush, *Methods Biochem. Anal.*, 13 (1965) 357.
13 J. Iwasa, T. Fujita and C. Hansch, *J. Med. Chem.*, 8 (1965) 150.
14 L. P. Hammett, *Physical Organic Chemistry*, McGraw-Hill, New York, 1940.
15 K.-G. Wahlund and I. Beijersten, *J. Chromatogr.*, 149 (1978) 313.
16 K.-G. Wahlund and A. Sokolowski, *J. Chromatogr.*, 151 (1978) 299.
17 P. R. Wells, *Linear Free Energy Relationships*, Academic Press, London, 1968.
18 M. S. Tute, in N. J. Harper and A. B. Simmonds (Editors), *Advances in Drug Research, Vol. 6*, Academic Press, London, New York, 1971, p. 1.
19 F. E. Norrington, R. M. Hyde, S. G. Williams and R. Wootton, *J. Med. Chem.*, 18 (1975) 604.
20 R. J. A. Walsh, K. R. H. Wooldridge, D. Jackson and J. Gilmour, *Eur. J. Med. Chem.*, 12 (1977) 495.
21 N. Tanaka, H. Goodell and B. L. Karger, *J. Chromatogr.*, 158 (1978) 233.
22 B. L. Karger, S. C. Su, S. Marchese and B.-A. Persson, *J. Chromatogr. Sci.*, 12 (1974) 678.
23 K. Sugden, G. B. Cox and C. R. Loscombe, *J. Chromatogr.*, 149 (1978) 377.
24 O. Sinanoğlu, in B. Pullmann (Editor), *Molecular Associations in Biology*, Academic Press, New York, 1968, pp. 427–445.
25 C. Horváth, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.
26 E. Tomlinson, *J. Chromatogr.*, 113 (1975) 1.
27 S. Eksborg and G. Schill, *Anal. Chem.*, 45 (1973) 2092.
28 I. M. Johansson and K.-G. Wahlund, *Acta Pharm. Suecica*, 14 (1977) 459.
29 J. M. Huen, R. W. Frei, W. Santi and J. P. Thevenin, *J. Chromatogr.*, 149 (1978) 359.
30 T. P. Moyer and N.-S. Jiang, *J. Chromatogr.*, 153 (1978) 365.
31 B. Mellström and G. Tybring, *J. Chromatogr.*, 143 (1977) 597.

32 C. Hansson, G. Agrup, H. Rorsman, A.-M. Rosengren, E. Rosengren and L.-E. Edholm, *Chromatogr.*, 162 (1979) 7.
33 M. T. Gilbert and R. A. Wall, *J. Chromatogr.*, 149 (1978) 341.
34 C. P. Terweij-Groen, T. Vahlkamp and J. C. Kraak, *J. Chromatogr.*, 145 (1978) 115.
35 P.-O. Lagerström and B.-A. Persson, *J. Chromatogr.*, 149 (1978) 331.
36 E. Tomlinson and S. S. Davis, *J. Colloid Interface Sci.*, (1979) in press.
37 T. Fujita, J. Iwasa and C. Hansch, *J. Amer. Chem. Soc.*, 86 (1964) 5175.
38 S. S. Davis, T. Higuchi and J. H. Rytting, *Advan. Pharm. Sci.*, 4 (1974) 73.
39 A. Packter and M. Donbrow, *Proc. Chem. Soc.*, (1962) 220.
40 G. I. Mukhayer, *Ph.D. Thesis*, University of Aston, 1974.
41 R. M. Diamond, *J. Phys. Chem.*, 67 (1967) 2513.
42 T. Higuchi, A. Michaelis and J. H. Rytting, *Anal. Chem.*, 43 (1971) 287.
43 R. F. Rekker, *The Hydrophobic Fragmental Constant*, Elsevier, Amsterdam, 1977.
44 Cs. Horváth, W. Melander and I. Molnár, *Anal. Chem.*, 49 (1977) 142.
45 S. C. Valvani, S. H. Yalkowsky and G. L. Amidon, *J. Phys. Chem.*, 80 (1976) 829.
46 R. C. Weast (Editor), *Handbook of Chemistry and Physics*, Chemical Rubber Co., Cleveland, Ohio, 50th ed., 1969, p. F-28.
47 A. L. Vierk, *Z. Anorg. Chem.*, 261 (1950) 283.
48 E. Tomlinson, C. M. Riley and T. M. Jefferies, (1979) in preparation.
49 S. S. Davis, G. Elson, E. Tomlinson, G. Harrison and J. C. Dearden, *Chem. Ind. (London)*, No. 16 (1976) 677.
50 E. Tomlinson, S. S. Davis and G. I. Mukhayer, in K. L. Mittal (Editor), *Solution Chemistry of Surfactants, Vol. 1*, Plenum Press, New York, 1979, pp. 3–343.
51 J. C. Kraak and J. F. K. Huber, *J. Chromatogr.*, 102 (1974) 333.
52 G. Prukop and L. B. Rogers, *Separ. Sci. Technol.*, 13 (1978) 59.
53 H. Colin, J. C. Diez-Masa, G. Guiochon, T. Czajkowska and I. Miedziak, *J. Chromatogr.*, 167 (1978) 41.
54 E. Tomlinson, H. Poppe and J. C. Kraak, *J. Pharm. Pharmacol. Suppl.*, 28 (1976) 43P.
55 W. Melander, D. E. Campbell and C. Horváth, *J. Chromatogr.*, 158 (1978) 215.
56 J. H. Knox and G. Vasvari, *J. Chromatogr.*, 83 (1973) 181.
57 E. J. Kikta and E. Grushka, *Anal. Chem.*, 48 (1976) 1098.
58 E. Tomlinson, H. Poppe and J. C. Kraak, *J. Chromatogr.*, submitted for publication.
59 A. Diez-Cascon, A. Serra, J. Pascual, M. Gassiat and J. Algaiges, *J. Chromatogr. Sci.*, 12 (1974) 559.
60 R. R. Krug, W. G. Hunter and R. A. Grieger, *J. Phys. Chem.*, 80 (1976) 2335.
61 R. R. Krug, W. G. Hunter and R. A. Grieger, *J. Phys. Chem.*, 80 (1976) 2341.
62 G. I. Mukhayer and S. S. Davis, *J. Colloid. Interface Sci.*, 65 (1978) 210.
63 S. A. Riddick and W. B. Bunger, *Organic Solvents. Physical Properties and Methods of Purification*, Wiley-Interscience, New York, 1970.
64 R. J. Laub and J. H. Purnell, *J. Chromatogr.*, 161 (1978) 49.
65 K. Karch, I. Sebastian and I. Halász, *J. Chromatogr.*, 122 (1976) 3.
66 M. C. Hennion, C. Picard and M. Caude, *J. Chromatogr.*, 166 (1978) 21.
67 J. Hermansson, *J. Chromatogr.*, 152 (1978) 437.
68 H. Colin, N. Ward and G. Guiochon, *J. Chromatogr.*, 149 (1978) 169.
69 C. M. Riley, E. Tomlinson and T. M. Jefferies, *J. Pharm. Pharmacol., Suppl.*, 30 (1978) 64P.