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STRUCTURAL EFFECTS IN ENTHALPY/ENTROPY COMPENSATED AND NON-COMPENSATED BEHAVIOUR IN ION-PAIR REVERSED-PHASE HIGH-PERFORMANCE LIQUID–SOLID CHROMATOGRAPHY

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SUMMARY

This contribution describes a number of linear enthalpy/entropy compensations found with various ion-pair reversed-phase high-performance liquid–solid chromatographic (RP-HPLSC) arrangements. Similar plots for these systems and for those described by non-ion-pair arrangements can be described, and analysis of these compensations in functional group terms indicates that differences in compensation plots are probably due to differences in phase ratios. Time and hydrophobic normalisation, and other extrathermodynamic approaches have been employed to study the effect of organic modifier on phase selectivity in ion-pair systems. The observation of a concomitant behavioural effect by temperature and eluent composition on retention of solutes in such systems has been analysed according to previously described models and it is demonstrated that for ion-pair RP-HPLC there exists a structurally dependent non-compensated residue for the enthalpy of retention. It is suggested that these residues are responsible for observed perturbations in a general enthalpy/entropy compensation relationship for RP-HPLSC.

INTRODUCTION

Linear enthalpy/entropy compensation in liquid chromatographic systems has been reported in a number of recent studies[1–8]. Some findings[2,5,7,8] suggest that in reversed-phase high-performance liquid chromatography (RP-HPLC) a single compensation relationship can be used to describe retention of various solutes with a variety of eluents and stationary phases. Theory[2,9,10] suggests that a similar mechanism of retention (*i.e.*, solvophobic chromatography[9]) can be operating in these systems. Previously[5] we have demonstrated that linear enthalpy/entropy compensation can occur in ion-pair RP-HPLC using surfactants as pairing ions, although it has been suggested[11] that such regular behaviour will generally not be observed for ion-pair systems. A subsequent finding that in our systems changes in temperature and/or

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eluent type and composition have the same regular effect on retention, has led us to analyse our results in terms of the extrathermodynamic approach developed recently by Melander *et al.*[4] for considering those concomitant (*sic*) effects found in LC caused by environmental factors. Further, by employing simple linear free-energy relationships we describe in this present contribution the effect of such behaviour with respect to solute structure.

EXPERIMENTAL

Materials

Benzoic acid, monosubstituted benzoic and phthalic acids (X = 2,3,4-Cl; 2,3,4-NH_2; 2,3,4-NO_2; 2,3,4-OH and 2,3,4-CH_3), phenylacetic acid, cinnamic acid, tyrosine and 3,4-dihydroxyphenylalanine were of at least reagent grade and were obtained from BDH (Poole, Great Britain) and Fisons (Loughborough, Great Britain), 12-dien-Zn(II) was prepared[12] from zinc sulphate and dodecyldiethylenetriamine (Kodak, Liverpool, Great Britain). Alkylbenzyldimethylammonium chlorides (ABDAC) were as described previously[5], and sodium dodecyl sulphate (SDDS) was of "biochemical" grade (BDH). Water was double-distilled from an all glass still. All other chemicals were of AnalaR grade (Fisons), except for methanol, acetonitrile, propan-2-ol and tetrahydrofuran which were of HPLC grade (Rathburn, Peebles, Great Britain)..

Packing materials used were Spherisorb S5 ODS (Phase Separations, Queensferry, Great Britain) and Hypersil SAS and ODS (Shandon Southern, Runcorn, Great Britain).

Procedures

Using a custom built HPLC apparatus described previously[5], temperature and mobile phase effects were studied by immersion of the column in a thermostatically controlled (± 0.1°C) water-bath. Column packing and preparation, and measurement procedures were as described before[5]. Capacity factors, k, are the means of at least three separate determinations, and were calculated from $[(t/t_0) - 1]$, where t was solute retention time and t_0 the retention time of water enriched mobile phase.

Simple and multiple linear regressions were carried out using standard computer programs.

RESULTS AND DISCUSSION

Thermodynamics

The dependence of solute capacity factors, k, on temperature in a reversed-phase LC system may be given by[1]

$$\kappa = -\frac{\Delta H}{2.3RT} + \frac{\Delta S}{2.3R} + \frac{\log \psi}{2.3} \qquad (1)$$

where κ is the logarithmic form of the capacity factor and ψ is the phase ratio. Thus the enthalpy change, ΔH, can be assessed from a plot of κ *versus* T^{-1}, which will be linear

if ΔH is constant. The entropy change, ΔS, can be determined only if ψ is known. Fig. 1 gives some representative Van 't Hoff plots obtained in the present study with various solutes, pairing ions, eluents and stationary phases. In all cases regular[11] behaviour is found with an increase in temperature causing a decrease in both retention and selectivity over the studied temperature range. Table I gives the found enthalpies of retention and results are seen to be consistent with a previous finding[5] that generally with ion-pair systems higher values are found compared with those measured in non-ion-pair LC.

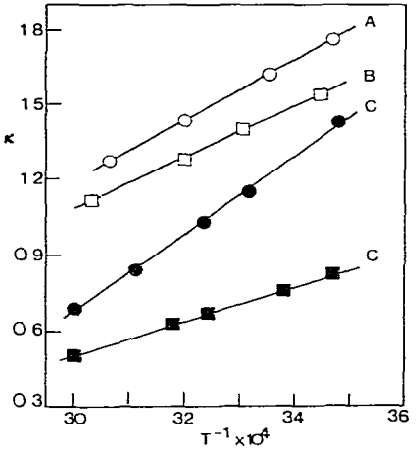

Fig. 1. Van 't Hoff plots for various solutes in a number of ion-pair HPLC arrangements. Key: A, B and C are tyrosine, 4-toluic acid and benzoic acid, respectively; closed squares, open squares, closed circles and open circles are respectively the phase systems 1–4 given in Table I.

Extrathermodynamics

(i) Linear enthalpy/entropy compensation

Physicochemical processes occurring in aqueous environments are often characterised by linear enthalpy/entropy compensation[10,13]. Leffler and Grunwald[10] have argued that to identify a single unique mechanism 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 either a medium effect, or, as for the present study, by a change in solute(s) structure(s)]. Using recommended coordinates[14,15] such compensation effects in reversed-phase LC are well described[2] by

$$\kappa_T = -(\Delta H/2.3R)\cdot(1/T - 1/\beta) - \Delta G_\beta/2.3R\beta + \log \psi/2.3 \quad (2)$$

where β is the enthalpy/entropy proportionality factor[10], and which has units of absolute temperature. Such an extrathermodynamic relationship can be described[5,8] in terms of functional group contributions by

$$\tau_T = -[\Delta(\Delta H)/2.3R]\cdot(1/T - 1/\beta) - \Delta(\Delta G_\beta)/2.3R\beta \quad (3)$$

TABLE I

ENTHALPIES OF RETENTION OF SIMPLE SOLUTES IN VARIOUS ION-PAIR RP-HPLC ARRANGEMENTS

$-\Delta H$ values are in kJ mol^{-1}; κ = log capacity factor at 40°C (interpolated values from Van 't Hoff plots).

Solute	*Phase system*	*1*		*2*		*3*		*4*					
	Stationary	*Hypersil SAS*		*Hypersil SAS*		*Spherisorb ODS**		*Hypersil ODS*					
	Modifier	*Acetonitrile, ϕ = 0.25*		*Acetonitrile, ϕ = 0.25*		*Acetonitrile, ϕ = 0.20*		*Propan-2-ol, ϕ = 0.02 (a), 0.04 (b)*					
	Pairing ion	*C14BDAC, $1 \cdot 10^{-3}$ mol dm^{-3}*		*12-dien-Zn(II), ($1 \cdot 10^{-3}$ mol dm^{-3})*		*C13BDAC ($5 \cdot 10^{-4}$ mol dm^{-3})*		*SDDS, $2.5 \cdot 10^{-4}$ mol dm^{-3} (i), $5 \cdot 10^{-4}$ mol dm^{-3} (ii)*					
		$-\Delta H$	κ	$-\Delta H$	κ	$-\Delta H$	κ	*(i)*	$-\Delta H$	κ	*(ii)*	$-\Delta H$	κ
Benzoic acid		16.5	0.70	18.1	0.90	34.5	1.04						
4-Aminobenzoic acid		10.0	0	14.2	0.46	20.5	0.27						
4-Toluic acid		20.6	0.99	21.3	1.28	40.1	1.36						
2-Phthalic acid		17.0	0.76	28.9	1.75								
3-Phthalic acid		21.6	0.77	27.8	1.19								
4-Phthalic acid		15.8	0.59	23.3	0.85								
Tyrosine								(a)	16.3	0.97	(a)	20.7	1.50
								(b)	15.1	0.90	(b)	23.2	1.48
3,4-Dihydroxy-phenylalanine								(a)	14.4	0.72	(a)	18.5	1.24
								(b)	13.0	0.65	(b)	16.8	1.23

* From ref. 5, where values for other substituted benzoic and cinnamic acids may be found.

where

$$\tau = \kappa_j - \kappa_i \tag{4}$$

and *i* and *j* refer to solutes differing by a functional group. Fig. 2 shows enthalpy/entropy plots according to eqn. 2 (ΔH–ΔG coordinates) for a number of ion-pair and non-ion-pair RP-HPLC systems determined here and elsewhere[5,8]. The remarkable feature of these plots is that apart from the 12-dien-Zn(II) system all arrangements show (according to theory[10,14,15]), linear enthalpy/entropy compensation behaviour. Similar slopes can be described for all these systems, although each plot is discrete from the others. If these displacements are due to differences in phase volume ratio then use of eqn. 3 to analyse the data in terms of group contributions should give similar slope and intercept coefficients for all systems. Eqns. 5–9 (Table II) comprise such an analysis, and indeed show both that slope coefficients are similar, and that in all cases intercept values (τ ordinate) approximate to zero. Although using 12-dien-Zn(II) as pairing ion these coefficients approximate to these found (Table II) in other RP systems, the very poor correlations coefficient suggest that here irregular[11] non-solvophobic behaviour is occurring.

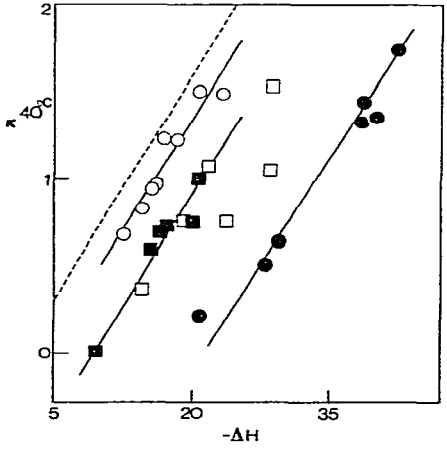

Fig. 2. Relationships between log capacity factor, κ, and enthalpies of retention, ΔH (kJ mol^{-1}), in ion-pair and non-ion-pair HPLC arrangements. Key: the dashed line is the regression line for a non-ion-pair system[8] using alkylsilicas as stationary phases (Table II), and data points are as for Fig. 1.

(ii) Linear free-energy relationships

Although Schoenmakers *et al.*[16] have theorised that the capacity ratio of a solute will vary quadratically with the volume fraction of organic modifier, ϕ, in a binary mixture eluent, it is often found that over a reasonably wide ϕ range that solute retention can be described by a linear function[17] of volume fraction; which can be given by

$$\kappa = \kappa_w + B\phi \tag{10}$$

where subscript w indicates on capacity value extrapolated to 100% aqueous eluent

TABLE II

FUNCTIONAL GROUP ENTHALPY/ENTROPY COMPENSATION RELATIONSHIPS ACCORDING TO EQN. 3

All data refer to an analytical temperature of 40°C and where necessary have been recalculated from the original measurements. n = Number of data points; r = correlation coefficient.

Solutes	*Phase system*	*Slope coefficient*	*Intercept coefficient*	*n*	*r*	*Eqn.*	*Ref.*
3-, 4-Monosubstituted benzoic and phthalic acids	Hypersil SAS; acetonitrile–acetate buffer (ϕ = 0.25); $1 \cdot 10^{-3}$ mol dm^{-3} C14BDAC pairing ion	−7.5	−0.11	5	0.922	5	This study
3-, 4-Monosubstituted benzoic and phthalic acids	Hypersil SAS; acetonitrile–acetate buffer (ϕ = 0.25); $1 \cdot 10^{-3}$ mol dm^{-3} 12-dien-Zn(II) pairing ion	−7.2	−0.17	5	0.834	6	This study
3-, 4-Monosubstituted benzoic acids	Spherisorb ODS; acetonitrile–phosphate buffer (ϕ = 0.20); $5 \cdot 10^{-4}$ mol dm^{-3} C13BDAC pairing ion	−6.8	0.04	7	0.976	7	5
3-, 4-Monosubstituted alkylbenzoates	LiChrosorb alkylsilicas; methanol–water (ϕ = 0.60–0.80)	−7.7	0.02	28	0.934	8	8
2-, 3-, 4-Monosubstituted alkyl benzoates	LiChrosorb alkylsilicas; methanol–water (ϕ = 0.60–0.80)	−9.1	0.03	40	0.949	9	8

and B is the slope coefficient. The effects of increasing the concentrations of four organic modifiers, *i.e.*, methanol acetonitrile, propan-2-ol and tetrahydrofuran, on the retention of a number of substituted benzoic acids in their ionized form as ion pairs, using a constant low concentration of alkylbenzyldimethylammonium chloride as pairing ion and Hypersil ODS as stationary phase are given by eqns. 11–68 (see Table III), using eqn. 10 as the analytical function. For all relationships the correlation between κ and ϕ was significant at the $\alpha = 0.001$ level, save with polar functions studied with tetrahydrofuran as modifier. In only one case (eqn. 55, Table III), could the introduction of a quadratic term[16] improve the correlation significantly. In all cases solute retention decreases (negative slope coefficients) with modifier. The values of κ_w and B are dependent upon substituent character, with values of B reflecting not only the effect of the organic modifier type on solute retention but also solute–solute and solute (ion-pair)–solvent interactions. Hence these coefficients may be taken to be a measure of the average solvation number of the formed ion pair in the solvent[18], with highest values of B being obtained with organic modifiers having the greatest extracting ability for the solute ion pairs. Based on benzoic acid behaviour these extracting abilities are given by: propan-2-ol (-10.9) > tetrahydrofuran (-9.3) > acetonitrile (-7.9) > methanol (-4.7). Clearly such values could form the basis of an elutropic series for solvents used in ion-pair reversed-phase high-performance liquid–solid chromatography (RP-HPLSC).

To examine the effect of solute structure on the coefficients of eqn. 10, it is convenient to introduce two group contribution terms, *viz.*

$$\tau_w = \kappa_{w_j} - \kappa_{w_i} \tag{69}$$

and

$$\Delta B = B_j - B_i \tag{70}$$

such that by combining eqns. 4, 10, 69 and 70 it follows that:

$$\tau = (\Delta B)\,\phi + \tau_w \tag{71}$$

These terms can now be related to other extrathermodynamic terms used to describe functional group physicochemical properties, and since retention in these systems can be regarded[5,9] as being controlled by both electrostatic and solvophobic forces, these two chromatographic terms have been correlated using regression analysis to group hydrophobicity and electronic parameters, *viz.*

(1) Slope coefficient term, ΔB

(a) Methanol

$\Delta B = 1.1\pi - 0.36$ $\quad n = 10,\ r = 0.922$ (72)

$\Delta B = 0.098\pi - 056\sigma - 0.22$ $\quad n = 10,\ R = 0.965$ (73)

(b) Propan-2-ol

$\Delta B = -2.98\pi - 0.51$ $\quad n = 8,\ r = 0.901$ (74)

$\Delta B = -2.62\pi - 1.39\sigma - 0.22$ $\quad n = 8,\ R = 0.951$ (75)

TABLE III

RELATIONSHIPS BETWEEN κ AND ORGANIC MODIFIER VOLUME FRACTION ACCORDING TO EQN. 10

Chromatographic details: stationary phase, Hypersil ODS; mobile phase modifier, tetradecylbenzyldimethylammonium chloride ($5 \cdot 10^{-4}$ mol dm^{-3}); pH 7.5 ($2.5 \cdot 10^{-2}$ mol dm^{-3} K_2HPO_4); 30°C.

Benzoic acid ring substituent	*Organic modifier*															
	Methanol				*Propan-2-ol*				*Acetonitrile*				*Tetrahydrofuran*			
	$-B$	κ_w	τ_w	*Eqn.*	$-B$	κ_w	τ_w	*Eqn.*	$-B$	κ_w	τ_w	*Eqn.*	$-B$	κ_w	τ_w	*Eqn.*
H	4.74	2.94		(11)	10.9	3.39		(26)	7.94	2.80		(39)	9.29	2.96		(54)
2-NH_2	3.21	1.43	−1.51	(12)	6.2	1.43	−1.84	(27)	4.54	1.24	−1.56	(40)	6.46	1.44	−1.52	(55)
3-NH_2	3.84	1.84	−1.10	(13)	8.1	2.10	−1.19	(28)	5.55	1.67	−1.13	(41)	7.36	1.92	−1.04	(56)
2-NH_2	5.22	3.10	0.16	(14)	11.2	3.38	−0.01	(29)	8.18	2.88	0.08	(42)	9.73	3.08	0.12	(57)
4-OH	4.22	2.20	−0.74	(15)	9.18	2.45	−0.94	(30)	5.27	1.58	−1.22	(43)	7.39	2.12	−0.84	(58)
3-OH	5.03	2.82	−0.12	(16)	11.2	3.20	−0.19	(31)	7.18	2.34	−0.46	(44)	7.71	2.93	−0.03	(59)
4-NO_2	5.38	3.44	0.50	(17)	12.9	4.16	0.67	(32)	8.62	3.36	0.56	(45)	15.3	5.19	2.23	(60)
3-NO_2	5.51	3.53	0.59	(18)	13.0	4.16	0.67	(33)	9.07	3.48	0.68	(46)	17.4	5.73	2.87	(61)
2-NO_2	4.53	2.75	−0.19	(19)	10.6	3.10	−0.29	(34)	7.26	2.64	−0.16	(47)	8.94	2.77	−0.19	(62)
4-CH_3	5.35	3.53	0.59	(20)	12.3	4.05	0.66	(35)	9.38	3.49	0.69	(48)	14.9	4.74	0.78	(63)
3-CH_3	5.48	3.63	0.69	(21)	12.3	4.04	0.65	(36)	9.93	3.66	0.86	(49)	14.7	4.67	0.71	(64)
2-CH_3	4.71	3.02	0.06	(22)	10.2	3.19	−0.20	(37)	7.89	2.79	−0.01	(50)	8.60	2.73	−0.23	(65)
4-Cl	6.11	4.14	1.20	(23)					10.6	4.10	1.30	(51)	13.0	4.54	1.60	(66)
3-Cl	6.00	4.10	1.16	(24)					10.4	4.08	1.28	(52)	13.1	4.55	1.61	(67)
2-Cl	4.74	2.85	−0.09	(25)	10.5	3.29	−0.10	(38)	8.67	3.06	0.26	(53)	9.03	2.85	−0.11	(68)

(c) Acetonitrile

$$\Delta B = -1.80\pi - 0.68 \qquad n = 10,\ r = 0.901 \quad (76)$$

$$\Delta B = -1.74\pi - 0.28\sigma - 0.63 \qquad n = 10,\ R = 0.811 \quad (77)$$

(d) Tetrahydrofuran

$$\Delta B = -4.15\pi - 2.50 \qquad n = 10,\ r = 0.800 \quad (78)$$

$$\Delta B = -3.45\pi - 2.83\sigma - 2.17 \qquad n = 10,\ R = 0.877 \quad (79)$$

where n refers to the number of (3- and 4-substituted) solutes and where R is the multiple correlation coefficient, and π and σ are the Hansch water/octan-1-ol group hydrophobicity constant[19] and the Hammett group electronic term[20] respectively. For the alcoholic organic modifiers the slope coefficient is seen to be controlled primarily by hydrophobic effects, although there is a significant electronic contribution (eqns. 73 and 75). For acetonitrile and tetrahydrofuran the dependency of the slope coefficient on substituent hydrophobicity is reduced, presumably due to specific solvation effects, and the fact that ion pairs retain some polarity[21].

(2) The intercept term, τ_w. The suggestion has been made[22–24] that the intercept value of eqns. 10 and 71 may be used as hydrophobicity parameters *per se* for use in, for example, drug design models and the estimation of bulk phase liquid–liquid distribution coefficients. Elsewhere[25] we have demonstrated that for non-ion-pair chromatography such a suggestion is tenable, and it is thus of interest to examine its validity for ion-pair arrangements. Thus:

(a) Methanol

$$\tau_w = 1.13\pi + 0.23 \qquad n = 10,\ r = 0.951 \quad (80)$$

$$\tau_w = 1.04\pi + 0.51\sigma + 0.21 \qquad n = 10,\ R = 0.990 \quad (81)$$

(b) Propan-2-ol

$$\tau_w = 1.33\pi + 0.16 \qquad n = 8,\ r = 0.945 \quad (82)$$

$$\tau_w = 1.20\pi + 0.51\sigma + 0.06 \qquad n = 8,\ R = 0.976 \quad (83)$$

(c) Acetonitrile

$$\tau_w = 1.32\pi + 0.21 \qquad n = 10,\ r = 0.912 \quad (84)$$

$$\tau_w = 1.22\pi + 0.40\sigma + 0.13 \qquad n = 10,\ R = 0.928 \quad (85)$$

(d) Tetrahydrofuran

$$\tau_w = 1.60\pi + 0.86 \qquad n = 10,\ r = 0.889 \quad (86)$$

$$\tau_w = 1.38\pi + 0.14\sigma + 0.27 \qquad n = 10,\ R = 0.939 \quad (87)$$

These relationships between τ_w and π are not as significant as those found[25] for non-ion-pair systems, although the higher correlations found using methanol and propan-2-ol indicate they are the most suitable organic modifier for obtaining such hydrophobicity indices for ionisable solutes. The correlations are significantly improved in all cases by the introduction of the electronic term, suggesting both solvation effects and a possible contribution of surface silanol groups[26] to retention.

(iii) Time and hydrophobicity normalisation

To compare functional group selectivity with different organic modifiers it is useful to use an organic solvent normalisation approach[27,28]. For the solutes and functions given in Table III, time normalisation can be made by choosing concentrations of organic modifiers which result in the same retention of a reference solute (benzoic acid), using methanol ($\phi = 0.50$). Differences in selectivities (group values) can be then obtained using eqn. 88

$$\tau_m = C\tau^* + D \tag{88}$$

which relates substituent values obtained using methanol, τ_m, to those obtained, τ^*, in "equivalent" mobile phases containing the other modifiers (where C and D are constants). Similarly for solutes we may write:

$$\kappa_m = E\kappa^* + F \tag{89}$$

Regression and correlation coefficients obtained using the systems described in Table III are given by eqns. 90–92:

acetonitrile:	$\kappa_m = 1.03\,\kappa^* - 0.09$	$n = 15, r = 0.980$	(90)
tetrahydrofuran:	$\kappa_m = 0.85\,\kappa^* + 0.07$	$n = 15, r = 0.921$	(91)
propan-2-ol:	$\kappa_m = 0.99\,\kappa^* - 0.01$	$n = 15, r = 0.992$	(92)

It may be seen (Figs. 3a–3c) that although propan-2-ol behaves similarly to methanol, the relationships for other modifiers are perturbed by the effect of polar functions (NO_2, NH_2 and OH), which are displaced significantly from the reference line of slope unity. However (Table IV), hydrophobic group values are shown to be organic modifier independent. These results may be explained within the context of solvophobic theory[9], which for functional groups can be given by

$$\tau = \Delta K_1 + K_2\,\Delta(\Delta[\mathrm{HA}]) \tag{93}$$

where ΔK_1 is related to the free energy of interaction of the functional group with the mobile phase due to van der Waal's interactions, K_2 is a constant related to the surface tension, γ, of the mobile phase and $\Delta(\Delta[\mathrm{HA}])$ is the change in hydrocarbonaceous surface contact between solute and stationary phase caused by the substituent, and is approximately equal to the hydrocarbonaceous surface area of the group[9]. Polar selectivity is influenced by both terms in eqn. 93, and hydrophobic selectivity is dominated by the second term, which may be given by $f\gamma \cdot \Delta(\Delta[\mathrm{HA}])$ since the surface tension is[9] proportional to K_2.

For two mobile phases we can show that

$$\tau^a - \tau^b = -\Delta K_1^a + \Delta K_1^b + f\gamma^a\,\Delta(\Delta[\mathrm{HA}]) - f\gamma^b\,\Delta(\Delta[\mathrm{HA}]) \tag{94}$$

where superscripts a and b refer to the two mobile phases. If γ^a equals γ^b then the hydrophobic content of the selectivity is normalized[27], such that now

$$\tau^a - \tau^b = \Delta K_1^b - \Delta K_1^a \tag{95}$$

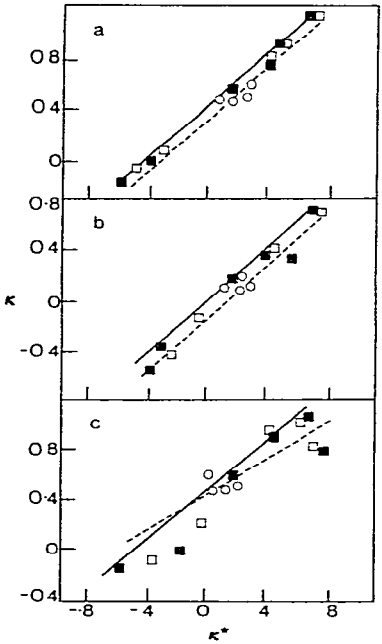

Fig. 3. Time normalised retention plots, showing the relationships between capacity factors of benzoic acids in eluents containing methanol (φ = 0.50), or (κ *) "equivalent" amounts of (a) propan-2-ol (φ = 0.255), (b) acetonitrile (φ = 0.275) and (c) tetrahydrofuran (φ = 0.265). The solid lines indicate a slope of unity and the dashed lines are regression lines according to eqns. 90–92. Key: 2-, 3- and 4-substituted solutes are open circles, open squares and closed squares, respectively.

and where for time and hydrophobic normalisation conditions:

$$\tau^a - \tau^b = \kappa_i^a - \kappa_i^b \tag{96}$$

Since Table IV gives hydrophobic group selectivity to be independent of modifier then this means that benzoic acid, as the ion pair, is behaving as a purely hydrophobic solute and that the hydrophobic contribution (eqn. 93) is normalised such that differences in mobile phase selectivities are only due to different solute–solvent interactions and will be restricted to polar functions. The perturbations of the relationships embodied in eqns. 78 and 79 are more apparent (Fig. 3) at lower equivalent organic

TABLE IV

HYDROPHOBIC GROUP VALUES WITH DIFFERENT ORGANIC MODIFIERS UNDER TIME NORMALIZATION CONDITIONS (FIG. 3), ACCORDING TO EQN. 88

Organic modifier		$\tau^*_{CH_3}$	τ^*_{Cl}
Type	*Concentration, ϕ*		
Methanol	0.500	0.30	0.53
Propan-2-ol	0.255	0.31	0.54
Acetonitrile	0.275	0.31	0.57
Tetrahydrofuran	0.265	0.28	0.57

TABLE V

CAPACITY FACTORS FOR SUBSTITUTED BENZOIC ACIDS UNDER IDENTICAL CONDITIONS USING EQUIVALENT CONCENTRATIONS OF PROPAN-2-OL (ϕ = 0.20) AND TETRAHYDROFURAN (ϕ = 20)

Benzoic acid substituent	*Capacity factor*	
	Propan-2-ol	*Tetrahydrofuran*
4-NH_2	1.4	1.9
3-NH_2	2.5	4.1
4-OH	3.1	6.2
3-OH	10.6	10.6
2-NO_2	10.6	9.1
2-NH_2	15.0	21.7
H	18.2	18.2

modifier concentrations where there is greater retention of the polar substituted benzoic acids. This effect is demonstrated by Table V which gives the retention of some polar substituted benzoic acids in equivalent mobile phases containing propan-2-ol and tetrahydrofuran. Although these two phase systems produce the same retention for the reference compound (benzoic acid), significant changes in selectivity and retention order are observed. These results indicate that if solute polarity is different, then separations may be improved by changing to an equivalent organic modifier (Fig. 4), containing a different functional group.

(iv) Eluent and temperature concomitant behaviour

Figs. 1 and 2, Table II and eqns. 72–79 indicate that the ion-pair systems studied here show regular[11] behaviour with respect to both temperature and eluent composition. This can be further appreciated by examination of Fig. 5, where, for example, a decrease in retention and selectivity with increased temperature can be compensated for by a decrease in organic modifier concentration. In terms of solute

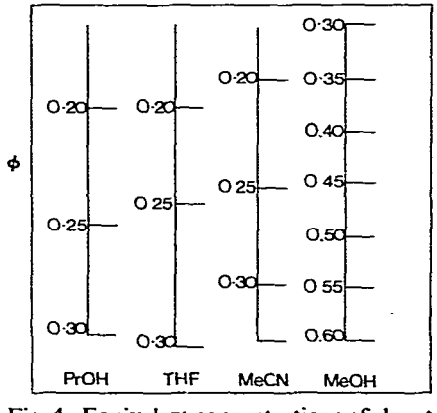

Fig. 4. Equivalent concentrations of eluent modifiers based on benzoic acid retention using ion-pair HPLC systems given in Table III. PrOH = Propan-2-ol; THF = tetrahydrofuran; MeCN = acetonitrile; MeOH = methanol.

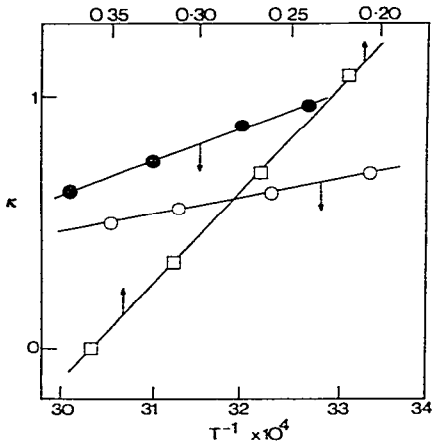

Fig. 5. Interrelationships between the effects of temperature (circles) at two different acetonitrile volume functions (closed ≙ 0.20; open = 0.25), and the effect of acetonitrile concentration (squares), at 30°C on the capacity factor of benzoic acid using ion-pair HPLC phase systems 1 and 3 (Table I).

structure it should therefore follow that the functional group terms of eqn. 71 are correlated with the group enthalpic contributions found for similar chromatographic arrangements. This is investigated by comparing the data for the enthalpies of retention obtained[5] using an aqueous acetonitrile eluent ($\phi = 0.20$) and Spherisorb ODS as stationary phase, and group terms obtained in this present study (Table III) using Hypersil ODS. Thus for 3- and 4-monosubstituted benzoic acid using ABDACs as pairing ions:

$$\Delta(\Delta B) = 26.8 \times 10^{-5}\,\Delta(\Delta H) - 0.03 \qquad n = 6,\ r = 0.977 \quad (97)$$

$$\tau_w = 13.6 \times 10^{-5}\,\Delta(\Delta H) + 0.06 \qquad n = 6,\ r = 0.984 \quad (98)$$

Considering the different stationary phases used and the precision in obtaining $\Delta(\Delta H)$ values these may be regarded as good correlations (*cf.*, Table II), and illustrate a concomitant effect in ion-pair RP-HPLC for temperature and organic modifier.

The dependence of κ on both solvent composition and temperature for RP systems can be analysed[4] to show whether ΔH is comprised of both a compensated, ΔH_c, and a non-compensated, ΔH_{nc}, portion at $\phi = 0$, *i.e.*

$$\Delta H(\phi) = \Delta H_{nc}(0) + \Delta H_c(0) \cdot f(\phi) \quad (99)$$

such that

$$\ln k = \frac{-\Delta H_c(0) \cdot f(\phi) - \Delta H_{nc}(0)}{RT} + \frac{\Delta H_c(0)[f(\phi) - 1]}{R\beta} + \frac{\Delta S(0)}{R} + \ln \psi \quad (100)$$

and where when $f(\phi) = 1 + \chi\phi$ (where χ is a constant, *i.e.*, eqn. 10 holds), then the dependence is given by[4]

$$\ln k = A_1\phi(1 + \beta/T) + A_2/T + A_3 \quad (101)$$

TABLE VI

MULTIPLE REGRESSION COEFFICIENTS ACCORDING TO EQN. 101 FOR ION-PAIR SYSTEMS GIVEN IN TABLE III USING ACETONITRILE (ϕ = 0.25) AS ORGANIC MODIFIER AND BENZOIC ACIDS AS SOLUTES

Solute	A_1	A_2	A_3	A_2/A_1
H	27.2	1799	4.47	66.1
4-NH_2	9.2	1058	2.88	115.0
3-OH	21.1	1546	3.88	73.2
4-OH	14.3	1428	4.05	99.9
4-NO_2	33.8	2008	4.49	59.4
4-CH_3	37.3	2087	4.72	56.0
4-Cl	41.3	2233	6.26	54.1
2-NH_2	30.2	1919	5.05	63.5
2-Cl	34.1	2118	3.09	62.1
2-CH_3	20.0	1323	2.74	66.2
2-NO_2	20.2	1464	3.04	72.5

where the meanings of A_1–A_3 are model dependent (Table I, ref. 4). Taking β for our systems as 525°K (which can be calculated from ref. 5 using eqn. 3), the data from Table III of this study have been analysed in terms of eqn. 101 at 30°C and at a ϕ value of 0.25, using multiple regression analysis. The multiple regression coefficients obtained are given in Table VI. Since the relationship between A_1 and A_2 may be given by

$$A_2 = 34.2A_1 + 835 \qquad n = 7, r = 0.990 \tag{102}$$

and the ratio A_2/A_1 is not constant (Table VI), this indicates[4] that for these ion-pair systems the enthalpy change upon retention is not exclusively compensated by ΔS and that A_1, A_2 and A_3 are given by $[\chi \Delta H_c\,(0)/2.2R\beta]$, $-[\Delta H_c\,(0) + \Delta H_{nc}\,(0)]/2.3R$ and $\Delta S\,(0)/2.3R + \ln \psi/2.3$ respectively. Since[4]

$$A_2 = \Delta H_{nc}(0)/2.3R - \beta A_1/\chi \tag{103}$$

analysis of our data (Table VI) gives for 3- and 4-monosubstituted benzoic acids a non-compensated enthalpy change residue of 15.4 kJ mol^{-1} (which compares with 15.9 kJ mol^{-1} found[4] for alkylbenzenes in a non-ion-pair RP system); and for 2-substituted benzoic acids a residue of 7.6 kJ mol^{-1}. The finding of this structurally dependent non-compensated residue appears to explain our previous findings (Table II) that *ortho* substituents perturb the general relationship between τ and Δ (ΔH), and may be a significant result[4] in elucidating retention mechanisms in reversed-phase liquid chromatography.

For non-ion-pair RP systems A_1, A_2 and A_3 are found[4] to be linearly related to the carbon number of the side chain of some *n*-alkylbenzene solutes, which, from Table II and previous discussion (eqns. 3 and 90), suggests that linear free-energy relationships exist for the present ion-pair systems between these regression terms and a solute hydrophobicity term. Fig. 6 shows this to be the case with excellent agree-

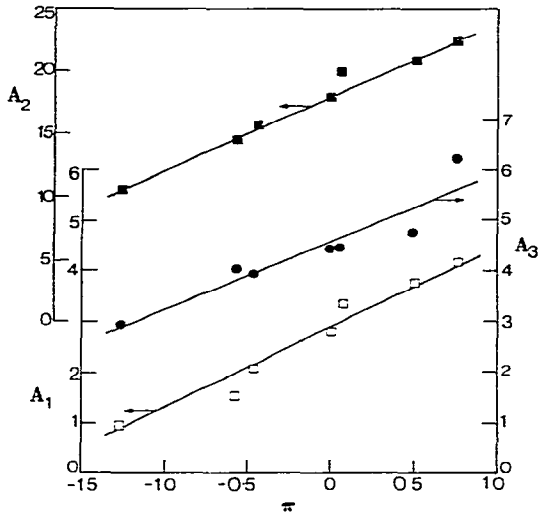

Fig. 6. Dependencies of multiple regression coefficients $A_1 \times 10^{-1}$, $A_2 \times 10^{-2}$ and A_3 from eqn. 101 on solute hydrophobicities as given by the Hansch π term[19]. Drawn lines are regression lines according to eqns. 104–106.

ment being found between the Hansch π term and A_2, the enthalpy dominated coefficient. For 3- and 4-substituted solutes we may formalise the relationships given in Fig. 6 by:

$$A_1 = 16.1\pi + 28.1 \qquad n = 7, r = 0.959 \qquad (104)$$

$$A_2 = 5.63 \times 10^2\,\pi + 1.80 \times 10^3 \qquad n = 7, r = 0.991 \qquad (105)$$

$$A_3 = 1.30\pi + 4.58 \qquad n = 7, r = 0.923 \qquad (106)$$

These data confirm the findings given in Table II which show enthalpy/entropy compensation effects determined in ΔH–ΔG coordinates. Further, eqn. 106 shows that there is a markedly reduced correlation between the entropy dominated term of eqn. 100, *i.e.*, A_3, and π.

CONCLUSIONS

(1) Solute structure effects on retention behaviour in various ion-pair HPLC arrangements can be well described using extrathermodynamic approaches. It is found that similar solute enthalpy/entropy linear compensations are demonstrated by various ion-pair and non-ion-pair systems, and that, in accord with solvophobic theory[9], analysis of these compensations in functional group terms, shows that differences in found compensation plots are probably due to the phase ratios of the various studied systems.

(2) Time and hydrophobic normalisation can be used for ion-pair LC to indicate differences in phase selectivities, and it is found that although methanol and propan-2-ol behave similarly, acetonitrile and tetrahydrofuran can have very different selectivities, indicating specific solvation effects.

(3) These solvation effects are seen to perturb the relationships described between functional group hydrophobicities and terms accounting for group retention behaviour with respect to changes in eluent type and composition.

(4) Finally, combining findings from this study (Table III) with our previous results[5] we have been able to determine whether the enthalpies of retention in ion-pair RP-HPLC are totally compensated by ΔS. This is demonstrated to be not the case, with, for 3- and 4-monosubstituted solutes a non-compensated residue of 15.4 kJ mol^{-1} (which is similar to that found[4] for non-ion-pair systems), and a value of 7.6 kJ mol^{-1} for 2-substituted solutes. These findings are suggested as a possible explanation of reported perturbations[5] in a general τ/Δ (ΔH) relationship found using *ortho*-substituted compounds.

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