

THERMODYNAMICS OF FUNCTIONAL GROUPS IN REVERSED-PHASE HIGH PERFORMANCE LIQUID–SOLID CHROMATOGRAPHY

E. TOMLINSON *, H. POPPE and J.C. KRAAK

Laboratory for Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, Amsterdam (The Netherlands)

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SUMMARY

The behaviour of functional groups having differing physicochemical characteristics have been examined in reversed-phase high performance liquid–solid chromatography using aqueous methanolic eluents. The effects of temperature, mobile phase organic modifier concentration and stationary phase on extrathermodynamic group values have been determined. Results fit into the framework of solvophobic theory with extrapolated group parameters being related to other extrathermodynamic terms. Group values exhibit linear enthalpy–entropy compensation behaviour as examined using ΔH – ΔG coordinates. A unique relationship between solute retention and the enthalpy of transfer for all solutes and phase systems examined is exhibited, and the overall study indicates that attempts to relate chromatographic retention data to drug liquid–liquid distribution is thermodynamically valid.

INTRODUCTION

Much recent attention has been given to the development of a priori methods for prediction of drug solution behaviour, and to the use of such methods in physicochemical and biological studies. Pharmaceutical science has focused frequently on the group contribution approach, (Davis et al., 1974), to realize these objectives in, for example, solution properties (activity coefficients), and liquid–liquid distribution parameters (partition coefficients). The rubric of this approach assumes that the free-energy of the process is comprised of the independent contributions of the component functional groups.

The advent of high performance liquid chromatography, (HPLC), brought about by

* Correspondence and present address: Subfaculty of Pharmacy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands.

the availability of reproducible systems and precision instruments has revived interest in the use of chromatography for providing physicochemical information of drug molecules. In particular, attempts have been made to relate directly or indirectly oil-water partition coefficients to HPLC data using either liquid-liquid phases, (Huber et al., 1972, 1973; Carlson et al., 1975; Henry et al., 1976; Mirrlees et al., 1976; Miyake and Terada, 1978; Unger et al., 1978; Unger and Feuerman, 1979), or liquid-solid phases, (McCall, 1975; Twitchett and Moffat, 1975; Molnár and Horváth, 1977, 1978; Tanaka and Thornton, 1977; Yamana et al., 1977; Baker, 1979; Baker et al., 1979; Löchmuller and Wilder, 1979; Chen and Horváth, 1979; Riley et al., 1979; Veith et al., 1979; Rittich et al., 1980). HPLC studies show a greater precision in data than approaches using thin-layer chromatographic methods, (Tomlinson, 1975), and generally relate some retention parameter to partition coefficients without examining the effect of environmental factors (phase composition, temperature, etc.).

In this present study we have examined the effect of various environmental factors on solute chromatographic retention while employing a functional group contribution approach. Chromatographic group contribution data can be described in thermodynamic terms, and this study has determined the contribution that individual moieties have to the free-energy changes taking place upon transfer from mobile to stationary phase in a number of systems. In addition, with regard to Franks' comment (Franks, 1975), that the free-energy term may hide more than it reveals due to possible enthalpy/entropy compensation effects, we have determined the group entropic and enthalpic contributions. This has been accomplished by measuring the column retention behaviour of up to 10 homologues of 10 series of substituted and unsubstituted alkylbenzoates in octyl and octadecyl chemically bonded reversed-phase systems using aqueous methanol eluents of varying composition, over a temperature range 20–50°C.

MATERIALS AND METHODS

Apparatus

A custom-made chromatograph was used throughout the study. Eluent was delivered from a 1 litre glass reservoir, which was thermostatted 5°C above the temperature of the column. Delivery was effected by a high pressure reciprocating membrane pump 0–325 bar, (Orlita DMP, 1515), with a flow-through manometer acting as a dampening device. Column loading of sample was made using a 10 μ l loop valve (Valco, model CB-6-UHPa-C-20 7000 PSIG), chosen for its operating specification at raised temperatures. The columns used were 30, 50 or 100 mm in length, and were constructed from 2.8 mm i.d. and 6.35 mm o.d. precision bore stainless steel (316) tubing. Detection was either by a differential flow-through refractometer, (Waters, model R401), with eluent as reference, or by a fixed wavelength (254 nm) ultraviolet detector (Waters, model 440). Chromatograms were recorded using a linear potentiometric chart recorder. Temperature control, $\pm 0.1^\circ\text{C}$, was maintained by totally immersing the column, end-fittings and capillaries, into a water-bath having a circulating flow facility, (Haake, type 21).

Chemicals

Octyl bonded chemically to silica, and octadecyl bonded to silica were used as model

stationary phases and were obtained from Merck, Darmstadt, F.R.G., (Lichrosorb RP-8 and RP-18, respectively), and had stated mean particle diameter size of 5 μm , with pore size and specific surface area estimated to be about 100 \AA and 400 m^2/g respectively.

Methyl to pentyl benzoates were commercially obtained, (Merck, Darmstadt, F.R.G.) and were of analytical grade. The hexyl to decyl unsubstituted and methyl to decyl substituted benzoates were synthesized and supplied by U.R. Tjaden of this laboratory, (Tjaden, 1976).

Double-distilled, deionised water was used throughout, and methanol was of analytical reagent grade, (Baker, A.C.S. specification). Potassium dichromate was of laboratory grade.

Procedure

Columns were packed by a balanced density slurry technique, (Tjaden et al., 1977), using tetrabromoethane–chloroform mixtures and packing pressures of 1000 atmospheres. Samples were dissolved in the appropriate mobile phase and injected generally as mixtures of homologues of each series.

Retention time was measured using a stop watch, and each reported value in this paper is the mean of at least 3 separate determinations. Throughout the course of the experiments, checks were made to ensure that a neighbouring homologue had no effect on the retention of any other homologue. This was performed by measuring the retention time of singly injected homologues. For very poorly retained solutes it was necessary to correct retention times for dwell times in the column-end capillaries. Freshly prepared aqueous methanol solutions of potassium dichromate were used to determine non-retained times.

RESULTS AND DISCUSSION

Functional group values

Functional group contribution towards retention, (Martin, 1950), may be defined as, (Riley et al., 1979):

$$\tau = \log r_{ji} = \log(\kappa_j \cdot \kappa_i^{-1}) = \log((t_{R_j} - t_{R_0})(t_{R_i} - t_{R_0})) \quad (1)$$

where κ and t_R are the capacity ratios and retention times of solutes j and i which differ by a functional group, r is the chromatographic selectivity coefficient and τ its logarithmic form, and t_{R_0} is the retention time of an unretarded tracer. In this present study, except where indicated, the reference solute, i , is taken as the ring unsubstituted ester. Capacity ratios may be related to solute distribution coefficients, K_d , by:

$$K_d = \kappa \cdot \phi^{-1} \quad (2)$$

where ϕ is the phase ratio. Hence the group term, τ , is equivalent to other substituent extrathermodynamic parameters, (Leffler and Grunwald, 1963), such as the ΔR_M TLC term, (Bush, 1965), or the Hansch π term, (Iwasa et al., 1965), derived from liquid–liquid distribution studies. Capacity ratios of 100 compounds comprising 10 homologous

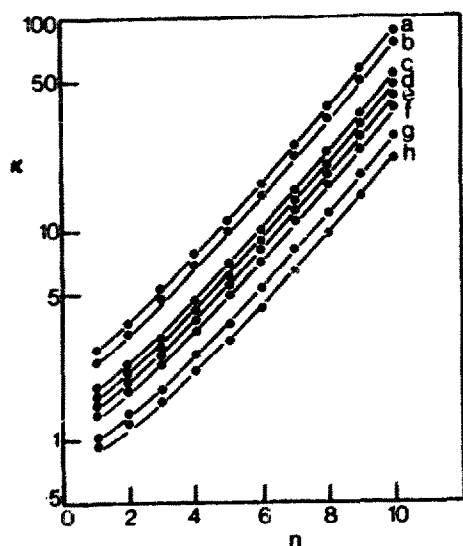


Fig. 1. Relationship between capacity ratio, κ , and carbon number of alkyl chain, n , for 8 homologous series of ring-substituted alkylbenzoates. Chromatographic conditions: octadecyl/methanol : water (80 : 20), 19.9°C. Key: a-h are 3-Cl, 4-Cl, 3-OCH₃, 4-OCH₃, 3-NO₂, 2-OCH₃ and 2-NO₂ respectively.

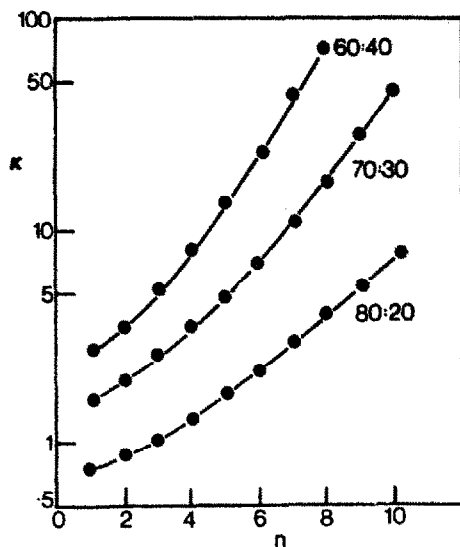


Fig. 2. Relationship between capacity ratio and homologue carbon number for 2-nitrobenzoates measured in an octyl/methanol : water system at 19.9°C at different organic modifier compositions.

series, (Materials), have been measured at 4 temperatures using 80 : 20 methanol : water as eluent. For 70 : 30 and 60 : 40 methanol : water compositions, generally only the retentions of the unsubstituted, the 2 methoxy- and the 2-nitro-substituted series have been determined to obtain methylene group values, and the retentions of the other C₂,

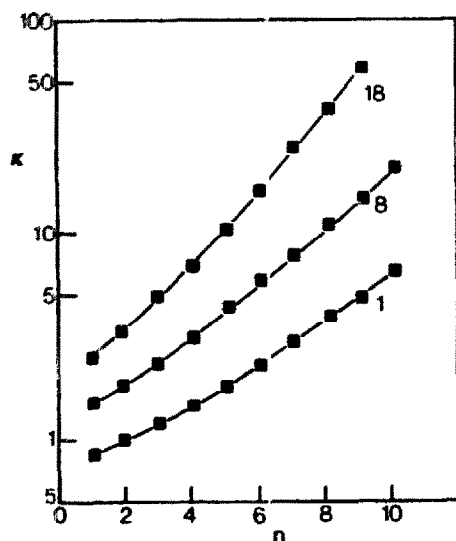


Fig. 3. Effect of chain length of alkylsilica stationary phase on retention of 3-chloroalkylbenzoates using methanol : water (30 : 20) as eluent, at ~20°C. (Stationary phase alkyl chain length is given next to each data line. Values for the methyl-bonded alkylsilica are from Tjaden, 1976.)

TABLE 1
EXTRATHERMODYNAMIC CHROMATOGRAPHIC GROUP VALUES τ

Functional group	Phase system									
	τ^a		τ_e^b		γ^b ($\tau = 0$) ($\text{mN} \cdot \text{m}^{-1}$)		τ^a	τ_e^b		γ^b ($\tau = 0$) ($\text{mN} \cdot \text{m}^{-1}$)
	Per cent methanol composition							Per cent methanol composition		
	80	70	60		60	70	80	70	60	
CH_2^c										
(1-2)	0.120	0.160	0.234			0.230	0.111	0.230	0.219	
(2-3)	0.124	0.164	0.240			0.233	0.134	0.233	0.240	
(3-4)	0.128	0.168	0.240			0.235	0.145	0.235	0.253	
(4-5)	0.132	0.181	0.243			0.241	0.158	0.241	0.271	
(5-6)	0.140	0.188	0.258			0.246	0.176	0.246	0.301	
(6-10)	0.146	0.188	0.261	1.076	20.1	0.250	0.182	0.250	0.305	18.9
2-OH ₃	-0.165	-0.225	-0.260	-0.936	17.3	-0.280	-0.211	-0.280	-0.315	15.6
3-OH ₃	0.040	0.050	0.063	0.226	17.7	0.060	0.051	0.060	0.080	18.0
4-OH ₃	0.015	0.025	0.035	0.177	23.1	0.040	0.022	0.040	0.050	22.6
2-NO ₂	-0.218	-0.265	-0.300	-0.883	12.2	-0.345	-0.315	-0.345	0.384	19.1
3-NO ₂	-0.024	-0.046	-0.068	-0.381	23.8	-0.052	-0.037	-0.052	-0.075	22.1
4-NO ₂	-0.052	-0.068	-0.096	-0.408	20.9	-0.090	-0.065	-0.090	-0.115	20.0
2-Cl	0.007	0.011	0.020	0.112	24.7	0.025	0.014	0.025	0.030	22.0
3-Cl	0.190	0.285	0.360	1.569	20.9	0.375	0.266	0.375	0.461	19.6
4-Cl	0.155	0.225	0.320	1.492	22.2	0.335	0.229	0.335	0.405	19.8

^a Each value derived from all homologues.

^b Extrapolated values using regression coefficients from Eqn. 4.

^c Numbers in parentheses refer to methylene position in ester alkyl-chain.

C6 and C7 homologues measured to determine other functional group contributions. This data set comprises some 1100 κ values (determined in triplicate), which have been a mean coefficient of variation of between 0.4 and 0.9% depending on the phase system. Excellent peak shape was obtained throughout, save for the strongly retained homologues of the chloroalkylbenzoates using high water eluent contents. Figs. 1–3 show the effects of solute structure, organic modifier composition and packing material alkyl chain length on retention, and Table 1 gives τ values for all substituents examined at 20°C.

Methylene group values are shown to be constant above an ester carbon chain-length of 5 (Table 1), whereas for the C1–C5 homologues there is a non-linear relationship between $\log \kappa$ and carbon number, such that τ_{CH_2} increases with chain length. Similar effects have been observed for alkylbenzoates on microspherical poly(styrene–divinylbenzene) gels, (Nakae and Muto, 1976), and for example, with *n*-alkanes (Karch et al., 1976), and *n*-alcohols (Karger et al., 1976). Such effects may be attributed in part to differences in hydrophobicity between methyl and methylene groups (Davis et al., 1974), effects of carboxy group hydration on lower homologues, and with a systematic error in determination of t_{R0} (Eqn. 4) (Berendsen and de Galan, 1980). Fig. 1 is typical for any one set of environmental conditions, hence, since ring substitution has no effect on methylene group values, group contributions for aromatic ring substituents are given by the vertical distance between $\log \kappa$ vs. homologue carbon number plots. An increase in eluent polarity leads to a rise in retention and relative retention, with both κ and τ_{CH_2} increasing (Fig. 2, Table 1). Horváth et al. (1976) have demonstrated that application of solvophobic theory (Sinanoglú, 1968) to reversed-phase high performance liquid–solid chromatography, (RP-HPLC), shows that for methanol–water eluents the relationship between retention and eluent surface tension may be given by

$$\ln \kappa = g + \frac{N \Delta A + 4.836N^{1/3}(\kappa^e - 1) V^{2/3} \cdot \gamma}{RT} \quad (3)$$

where g is a term which varies with organic modifier composition and type of solute; N , R , T and V are Avogadro's number, the gas constant, absolute temperature and average molar volume of the eluent, respectively; ΔA is the relative surface contact area of the solute molecule with the stationary phase, and can be indicated by molecular surface area; and κ^e may be defined as the ratio of the energy required to extend the planar surface of the solvent by the surface area of the added solute molecule. For methanol–water mixtures the term g in Eqn. 3 is related largely to the van der Waals component of the free-energy of interaction of the solute with the solvent, and can be given by (Horvath et al., 1976) $\Delta G_{\text{vdw}}/RT$. This will change with organic modifier composition and hence in group contribution terms we may recast Eqn. 3 as

$$\tau = \frac{\Delta(\Delta G)_{\text{vdw}} + N\gamma(\Delta A_j - \Delta A_i)}{2.3RT} \quad (4)$$

where $(\Delta A_j - \Delta A_i)$ is a group constant term describing the alteration in surface contact area of the solute with the stationary phase caused by a substituent. According to Eqn. 4 a plot of τ vs. γ should be linear for all functional groups, independent of group character

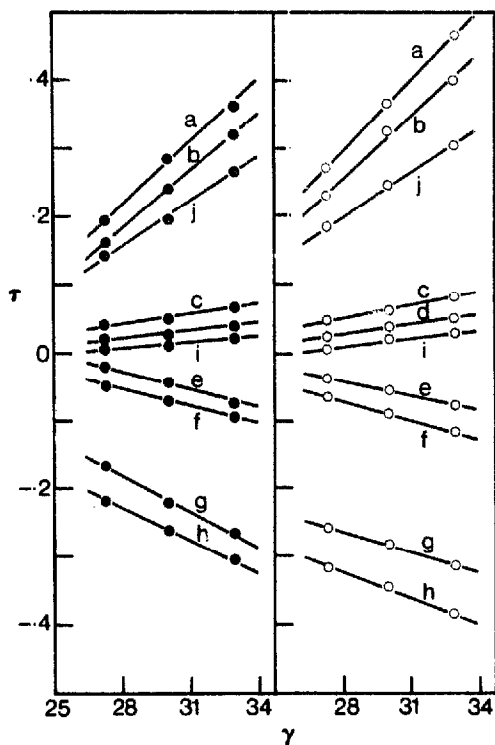


Fig. 4. Relationships between τ and mobile phase surface tension, γ , ($\text{mN} \cdot \text{m}^{-1}$), using octyl (closed datum points) and octadecyl (open datum points) stationary phases. Key: a–h as for Fig. 1, i and j are 2-Cl and CH_2 groups, respectively.

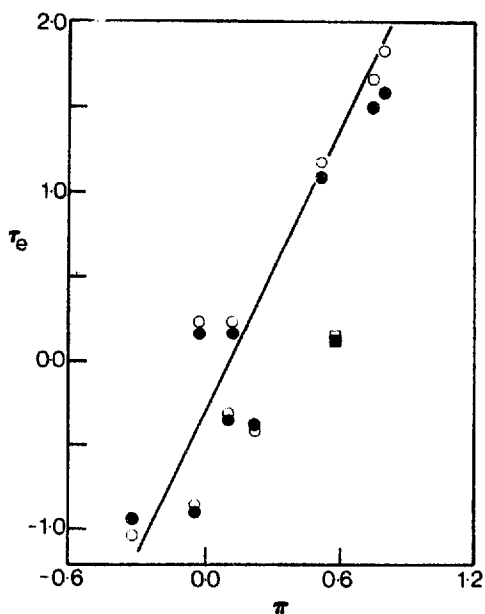


Fig. 5. Relationship between liquid-liquid distribution group values, π , (Fujita et al., 1965), and chromatographic τ_e values, determined by extrapolation using the regression coefficients of Eqn. 4 for each function, (Table 1). Closed and open data points are for RP-8 and RP-18 stationary phases, respectively, and squares are for the 2-chloro substituent. The line drawn is not a regression line but is intended to indicate a trend.

and, for example, stationary phase alkyl chain length. Fig. 4 shows this to be the case, with linear relationships existing between all substituents and eluent surface tension at 20°C . Using Eqn. 4 derived regression coefficients have been used to calculate two extrapolated values (see Table 1); that is, τ_e , which is a group contribution term at a hypothetical 100% eluent water composition; and $\gamma_{(\tau=0)}$, which corresponds to the abscissa intercept term in Eqn. 4 when τ values are set at zero. Omission of the 2-nitro- and 2-methoxy values (since these have an effect on the benzoate carboxy function) gives $\gamma_{(\tau=0)}$ values of 21.5 (S.D. = 2.4) and 20.4 (S.D. 1.7) $\text{mN} \cdot \text{m}^{-1}$, when using octyl and octadecyl stationary phases respectively. That all $\gamma_{(\tau=0)}$ values (Table 1) approximate to the surface tension of pure methanol at 20°C (i.e. $22.6 \text{ mN} \cdot \text{m}^{-1}$), means that no stationary phase selectivity then exists, which argues for the stationary phase behaving as the mobile phase (i.e. as pure methanol) due, apparently, to an adsorbed layer of methanol.

Hypothetical τ values at 100% water composition (τ_e), have been derived to provide a standard set of conditions for comparison with other group terms. Fig. 5 shows the

approximate relationship found between both sets of τ_e values and water/octan-1-ol π values (Fujita et al., 1965). The appropriate correlation equations (analyzed by least-squares regression) are given below, i.e.

$$\tau_e^8 = 2.30\pi - 0.33 \quad n = 9 \quad r = 0.914 \quad (5)$$

$$\tau_e^{18} = 2.64\pi - 0.34 \quad n = 9 \quad r = 0.915 \quad (6)$$

where n and r refer to the number of data points and the correlation coefficient, respectively. (The 2-chloro substituent is an obvious exception to these relationships, and has been omitted from the regression analyses. It should be appreciated that π values used in this study are obtained from literature $\log K_d$ values obtained for a phenoxyacetic acid series.) The τ_e terms are not well correlated with Hammett σ values ($r = 0.35$), but the latter can be used to improve the τ_e vs. π correlation, viz:

$$\tau_e^{18} = 2.70\pi - 0.60\sigma - 0.16 \quad n = 9 \quad R = 0.965 \quad (7)$$

$$\tau_e^8 = 2.42\pi - 0.65\sigma - 0.16 \quad n = 9 \quad R = 0.967 \quad (8)$$

where R is the multiple correlation coefficient. For Eqns. 7 and 8 both abscissa parameters are significant at the α , 0.01 level. The improvement in correlation found using σ values has not been found for ion-pair reversed-phase systems using Hypersil packing materials (Riley et al., 1979), and may reflect the presence of residual silanol groups with Lichrosorb compared to the highly silanized Hypersil surface.

Locke (1974) has concluded that selectivity in reversed-phase HPLC is independent of stationary phase alkyl chain length. Fig. 3 and the data for τ_{CH_2} given in Table 1 contradict this hypothesis, and are in agreement with other studies (Karch et al., 1976a and b; Berendsen and de Galan, 1980), that methylene group selectivity is affected by the nature of both the stationary phase and the eluent. Interestingly, this present study shows that at high organic modifier compositions this discrimination is blurred, which appears to reflect current theories of solute retention in RP-HPLC that describe selective adsorption of, for example, methanol onto the packing material.

Thermodynamics

The free-energy change, ΔG , during solute retention can be related to both the thermodynamic distribution constant for the process, and the capacity ratio using the van 't Hoff isotherm and Eqn. 2, i.e.

$$\Delta G = -RT \ln K_d = -RT \ln(\kappa \cdot \phi^{-1}) \quad (9)$$

Developing this into the van 't Hoff equation then for the effect of temperature on retention we may write (Melander et al., 1978)

$$\ln \kappa = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln \phi \quad (10)$$

Thus the enthalpy, ΔH , can be assessed from a plot of, for example, $\ln \kappa$ vs. T^{-1} , and if the enthalpy remains constant this will be a straight line. The entropy change, ΔS , caused by a solute can be determined only if the phase ratio is known. This is virtually impossible for brush-type reversed-phase stationary phases, (Löchmüller and Wilder, 1979). Analysis can be made, however, in group contribution terms. The theoretical basis (Conden et al., 1944; Martin, 1950) for the relationship between retention in distributing systems lies in the assumption that the free-energy change can be expressed as the sum of molecular group contributions (Pierotti et al., 1959), and hence if solute *i* is substituted by *n* groups *X* and *m* groups *Y*, etc., then for one mole of material

$$-RT \ln(\kappa \cdot \phi^{-1}) = \Delta G_i + n\Delta G_X + m\Delta G_Y + \dots \text{etc.} \quad (11)$$

and thus, for example, it follows from Eqn. 1 that

$$\tau = -(\Delta(\Delta G)_X)/(2.3RT) \quad (12)$$

and by combining Eqns. 1, 10 and 11, we obtain

$$\tau = -(\Delta(\Delta H))/(2.3RT) + \Delta((\Delta S))/R \quad (13)$$

To determine the energetics of functional groups the influence of temperature on solute retention has been measured between ~ 20 and 50°C for all phase systems having an octyl-bonded stationary phase, and in the 80 : 20 methanol : water/octadecyl system. Fig. 6

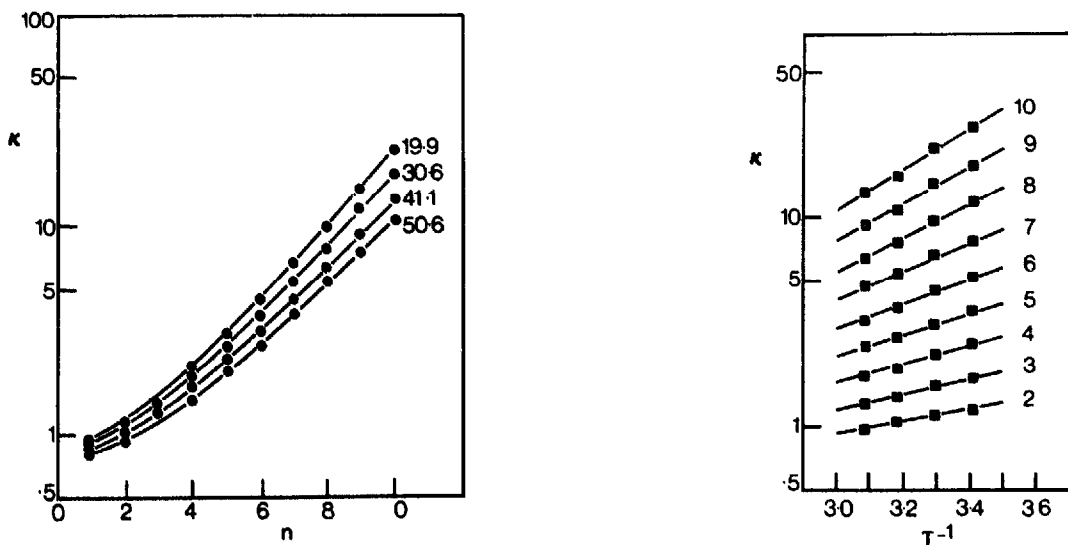


Fig. 6. Effect of temperature on the retention of 2-nitrobenzoates determined in an RP-18/MeOH : H₂O (80 : 20) system. Temperatures, ($^\circ\text{C}$), are given next to each data line.

Fig. 7. Van 't Hoff plots, (κ^{-1} vs. reciprocal absolute temperature, $^\circ\text{K}^{-1} \times 10^3$), for 2-methoxyalkylbenzoate homologues, determined in a RP-18/MeOH (80 : 20) system. Solute homologue number is given next to each data line.

TABLE 2
 ENTHALPIES OF SOLUTE TRANSFER DETERMINED IN AN OCTADECYL/METHANOL : WATER (80 : 20) PHASE SYSTEM

Function	$-\Delta H$ (kJ · mol ⁻¹)									
	Homologue number									
	10	9	8	7	6	5	4	3	2	1
2-OCH ₃	19.22	17.21	15.01	13.01	11.29	9.90	8.50		6.24	
3-OCH ₃	21.85	19.65	17.58	15.69	13.65	12.14	10.67			
4-OCH ₃	21.21	19.13	17.13	15.24	13.17					
2-NO ₂	18.73	16.73	15.22	13.00	11.74		8.52	9.38	5.65	
3-NO ₂	21.38	19.36	17.49	15.46	13.44	12.16				
4-NO ₂	19.72	17.84	15.98	14.08	12.10					
2-Cl	21.09	19.13	17.05	15.00	12.94					
3-Cl	23.25	21.35	19.41	17.50	15.31		11.50	9.99	7.91	6.51
4-Cl	22.94	21.04	18.97	17.00	15.02		11.01		7.20	6.81
H	20.61	18.54	16.63	14.66			9.14		6.23	

Omitted values indicate either that less than 4 temperatures were studied for that compound or that the regression coefficient for the $\ln \kappa$ vs. T^{-1} was less than 0.9.

describes the effect of temperature on retention of 2-nitroalkylbenzoates in the latter system. Similar profiles were obtained for all solute series in each phase system, and show that an increase in temperature causes a fall in both methylene group selectivity and solute capacity ratio. Fig. 7 describes van 't Hoff relationships for 2-methoxyalkylbenzoates and is typical of all systems. Table 2 gives the measured enthalpy changes for all solutes determined in the 80 : 20 methanol : water/octadecyl system (other values will be given as group contribution terms, below).

Table 2 shows that, in common with all literature values, the enthalpy value indicates an exothermic process over the studied temperature range. It has been argued (e.g. Scheraga, 1979) that the strength of hydrophobic effects should increase with temperature. This postulate is often not valid experimentally. For example, it has been observed recently (Tomlinson et al., 1979) that large organic ion complexation in water as reinforced by hydrophobic interactions (Tomlinson and Davis, 1980), is accompanied by a positive enthalpy and entropy change at temperatures below 15°C, but is associated with a negative enthalpy change and an increasingly less positive entropy change as temperature is raised to 60°C. Melander et al. (1978) have discussed this contradiction with respect to retention in RP-HPLSC, and conclude that there is a major inconsistency between the assignment of a positive sign for enthalpy with a hydrophobic effect, and the often observed behaviour of a fall in capacity ratio as temperature increases (i.e. a negative ΔH).

Enthalpy-entropy linear compensation

Physicochemical processes occurring in aqueous environments are often characterized by an enthalpy-entropy compensation effect (Leffler and Grunwald, 1963; Lumry and Rajender, 1970). Leffler and Grunwald 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)). Recently it has been shown, (Krug et al., 1976a and b), that enthalpy-entropy compensation behaviour should not be studied using ΔH - ΔS coordinates, since this can lead to artifacts caused not by true compensation between the two but by statistical effects. Rather, it is recommended, should regressions of functionalities between thermodynamic variables be carried out in a $\Delta H^{\text{Thm}} - \Delta G^{\text{Thm}}$ region, where T_{hm} refers to the harmonic mean temperature of the experiments, since, it is argued, unbiased estimates of the parameters will be obtained which will aid in proving the existence of linear relationships between ΔH and ΔS . Enthalpy entropy compensation is well expressed (Krug et al., 1976a) by

$$\Delta H = \beta\Delta S + \Delta G \text{ (at } T = \beta) = \beta\Delta S + \Delta G_{\beta} \quad (14)$$

which is consistent with the Gibbs equation, such that a linear relationship between ΔH and ΔG must exist, i.e.,

$$\Delta H = \lambda\Delta G + (1 - \lambda) \Delta G_{\beta} \quad (15)$$

where

$$\lambda = 1/(1 - T/\beta) \quad (16)$$

β is a proportionality factor as defined by Leffler and Grunwald (1963) and, having the dimensions of absolute temperature, has come to be termed the compensation temperature. Eqn. 14 can be rewritten (Leffler and Grunwald, 1963) to express the free-energy change measured at a fixed temperature T , (ΔG_T), as

$$\Delta G_T = \Delta H(1 - T/\beta) + T\Delta G_\beta/\beta \quad (17)$$

Hence by combining Eqns. 10 and 17 it can be shown (Melander et al., 1978) that for RP-HPLSC systems

$$\ln \kappa_T = -(\Delta H/R) \cdot (1/T - 1/\beta) - \Delta G_\beta/R\beta + \ln \phi \quad (18)$$

which in group contribution terms (Eqn. 13) we can show will become

$$\tau_T = -(\Delta(\Delta H)/2.3R) \cdot (1/T - 1/\beta) - \Delta(\Delta G_\beta)/R\beta \quad (19)$$

Using 35°C as the harmonic mean temperature, T_{hm} , all data for the present study (e.g. Table 2) have been examined using Eqn. 18. Values for $\kappa^{35^\circ\text{C}}$ have been obtained using the regression equations used to obtain ΔH values from $\ln \kappa$ vs. T^{-1} plots. Fig. 8 is the compensation plot for all unsubstituted and substituted heptylbenzoates using 80 : 20

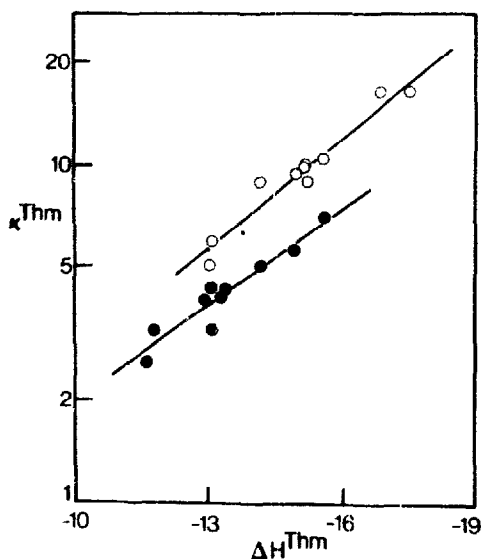


Fig. 8. Enthalpy-entropy compensation plot according to Eqn. 18 for heptylbenzoates showing capacity ratio, κ , vs. enthalpy, ΔH , ($\text{kJ} \cdot \text{mol}^{-1}$) found at the experimental harmonic mean temperature (35°C) for RP-8 (closed datum points), and RP-18 (open datum points), stationary phases and a methanol : water (80 : 20) eluent. Drawn lines are regression lines for each phase system.

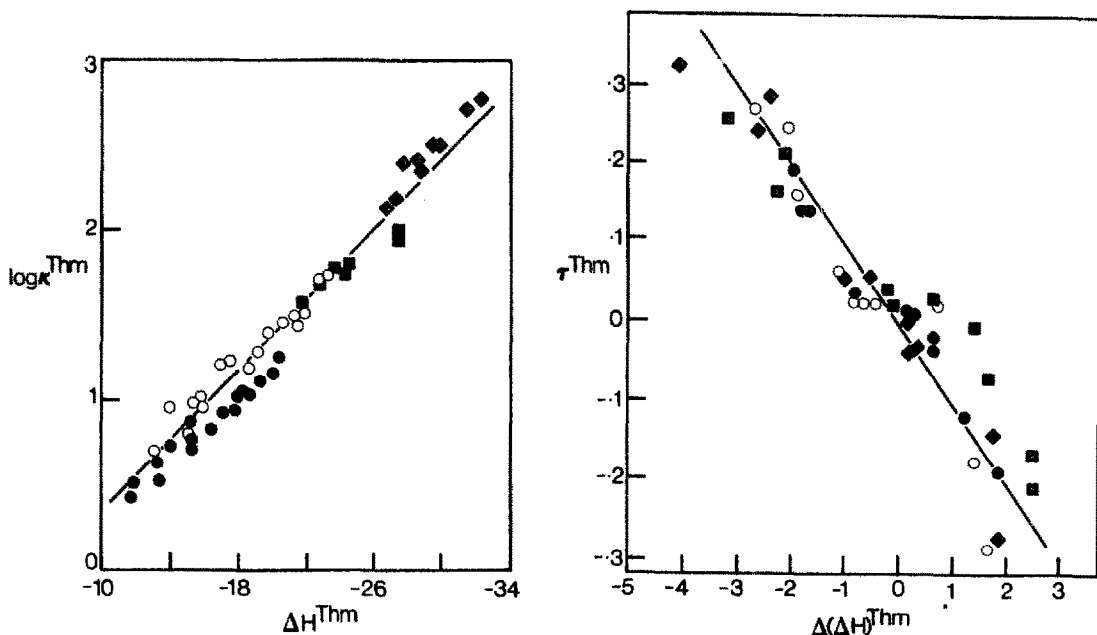


Fig. 9. Enthalpy-entropy compensation plot according to Eqn. 18 for substituted and unsubstituted hexyl-, heptyl- and decylbenzoates in various phase systems. Overlapping data points have been omitted for clarity. The drawn line is the regressed line for all data, as given by Eqn. 20. Key: stationary phase as for Fig. 8; circles, squares and diamonds refer to 80 : 20, 70 : 30 and 60 : 40 methanol : water eluents respectively.

Fig. 10. Compensation plot for functional groups according to Eqn. 19 for 10 substituents determined in 4 phase systems (Tables 1 and 3). The drawn regression line is as given by Eqn. 21. Key: as for Fig. 9.

methanol : water eluents and octyl and octadecyl stationary phases. Compensation behaviour occurs in both phases, with the octyl plot having a lower slope coefficient than for the octadecyl phase. Melander et al. (1978) have described similar compensation effects for 8 solutes using RP-HPLSC with octadecylsilica as the stationary phase, and either neat aqueous phosphate buffer (pH 2 or 7) or the same buffer containing 6% or 30% (v/v) acetonitrile as eluent. This same study analyzed data from other work for the retention of ketones and aldehydes in various phase systems, and although compensation behaviour could be described, β values were significantly lower in some systems indicating different retention behaviours. Viewing only the relationships given by Fig. 8 it is possible for the present study to propose similar differences in retention behaviours between the octyl and octadecyl systems. However, inclusion of ΔH^{Thm} and κ^{Thm} values for all the alkylbenzoates determined in all 4 phase systems (Fig. 9) shows both that this postulate would be invalid and that a general relationship exists in RP-HPLSC between solute retention and enthalpy for methanolic eluents irrespective of stationary phase chain length. Fig. 9 does not include all available values for clarity purposes. The relationship for all solutes is given by

$$\log \kappa^{35^\circ\text{C}} = -0.104\Delta H - 0.69 \quad n = 89 \quad r = 0.979 \quad (20)$$

TABLE 3
FREE-ENERGY, ENTHALPIC, ENTROPIC AND τ GROUP CONTRIBUTIONS

Functional group	Phase system							
	Octyl/methanol : water (60 : 40)				Octyl/methanol : water (70 : 30)			
	τ	$\Delta (\Delta G)$	$\Delta (\Delta H)$	$\Delta (\Delta S)$	τ	$\Delta (\Delta G)$	$\Delta (\Delta H)$	$\Delta (\Delta S)$
2-OCH ₃	-0.25	1.50	1.79	0.95	-0.18	1.06	2.50	4.62
3-OCH ₃	0.06	-0.35	-1.07	-2.32	0.04	-0.26	-0.30	-0.13
4-OCH ₃	0.06	-0.35	-0.62	-0.86	0.03	-0.18	0.66	2.72
2-NO ₂	-0.29	1.68	2.07	1.26	-0.22	1.22	2.45	4.01
3-NO ₂	-0.05	-0.29	0.19	0.32	-0.01	0.04	1.30	4.10
4-NO ₂	-0.05	-0.29	0.35	0.20	-0.08	0.45	1.57	3.64
2-Cl	0	0	0.20	0.65	0.02	-0.09	-0.07	0.06
3-Cl	0.33	-1.94	-4.10	-6.92	0.25	-1.48	-3.20	-5.57
4-Cl	0.29	-1.73	-2.35	-2.07	0.21	-1.22	-2.17	-3.09
CH ₂	0.24	-1.41	-2.53	-3.62	0.17	1.00	-2.32	-4.29

$\Delta (\Delta H)$ values are means of between 3 and 50 unique values depending on the functional group and the phase system. Each unique value being determined in triplicate.

$\Delta (\Delta G)$, $\Delta (\Delta H)$ and $\Delta (\Delta S)$ have units of $\text{kJ} \cdot \text{mol}^{-1}$, $\text{kJ} \cdot \text{mol}^{-1}$ and $\text{J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ respectively.

The variance between this relationship and that for other systems is due to the different phase ratios (Eqn. 18). Using group data (Eqn. 19) $\Delta(\Delta H)$, $\Delta(\Delta S)$ and $\Delta(\Delta G)$ values have been derived at $T_{\text{hm}} = 35^\circ\text{C}$ from the parent solute ΔH values, from extrapolated τ values and from the Gibbs equation respectively, and are given in Table 3. Fig. 10 is a plot of $\tau^{35^\circ\text{C}}$ vs. $\Delta(\Delta H)^{\text{Thm}}$ for all functional groups examined in 4 phase systems. The good relationship existing between the two is embodied in Eqn. 21, viz.

$$\tau^{35^\circ\text{C}} = -0.091 \Delta(\Delta H)^{\text{Thm}} - 0.01 \quad n = 40 \quad r = 0.949 \quad (21)$$

The generality of this equation for RP-HPLSC systems can be discerned from Table 4 which compares the regression coefficients of Eqn. 21 with similar relationships found by us for ion-pair RP-HPLSC systems. With a larger variety of functionalities and phase systems it should be possible to confirm and/or improve the coefficients of Eqns. 20 and 21, such that the predictive nature of both for describing solute physicochemical and chromatographic retention behaviour may be realized. Related to this aim will be an observation arising from the present study that τ and $\Delta(\Delta G)$ values from any one methanol : water/alkylsilica system are directly correlated with another. For example,

$$\tau_1 = 1.54 \tau_2 + 0.03 \quad n = 15 \quad r = 0.993 \quad (22)$$

and

$$\Delta(\Delta G)_{e'} = 1.11 \Delta(\Delta G)_{e''} - 0.20 \quad n = 10 \quad r = 0.998 \quad (23)$$

Phase system							
Octyl/methanol : water (80 : 20)				Octadecyl/methanol : water (80 : 20)			
τ	$\Delta (\Delta G)$	$\Delta (\Delta H)$	$\Delta (\Delta S)$	τ	$\Delta (\Delta G)$	$\Delta (\Delta H)$	$\Delta (\Delta S)$
-0.15	0.91	1.27	1.17	-0.18	1.07	1.49	1.19
0.03	-0.18	-0.91	-2.34	0.06	-0.28	-1.08	-2.63
0.01	0.83	0.11	0.09	0.02	-0.14	-0.58	-1.40
-0.20	1.19	1.87	2.20	-0.29	1.68	1.69	0.03
-0.02	0.11	0.60	-2.31	-0.02	0.11	-0.81	-2.99
-0.04	0.22	0.60	1.22	-0.02	0.14	0.71	1.84
0.01	-0.03	0.21	0.77	0.02	-0.09	-0.46	-1.21
0.18	-1.08	-2.02	-3.05	0.27	-1.58	-2.76	-3.85
0.14	-0.83	-1.65	-2.65	0.24	-1.40	-2.08	-3.18
0.13	-0.77	-1.72	-3.07	0.16	-0.97	-1.94	-3.15

where subscripts 1 and 2 refer to octadecyl/methanol : water (60 : 40) and octadecyl/methanol : water (80 : 20) systems respectively, and e' and e'' refer to extrapolated τ values (Table 1) computed using Eqn. 4, for octadecyl and octyl alkylsilicas respectively. Such relationships are similar to those first observed by Collander (1951) who, when correlating partition coefficients measured in different but related solvent pairs, found a logarithmic relationship between the K_d values for a series of solute analogues.

CONCLUSIONS

This work shows clearly a single unique relationship between alkylbenzoate retention and enthalpies of transfer from aqueous methanol eluents to alkylsilica stationary phases. The linear plot described by Fig. 9 and Eqn. 20 can be transformed into $\Delta H^{\text{Thm}} - \Delta G^{\text{Thm}}$ coordinates, having a slope related (Eqn. 16) to the compensation temperature, β , by

$$\beta = T^{\text{hm}} / (1 - \lambda^{-1}) \quad (24)$$

For the present study this gives a compensation temperature of 794°K which both compares with values of between 596 and 743°K described for other reversed-phase systems (Melander et al., 1978; Vigh and Varga-Puchony, 1980) and falls within or approaches their 95% confidence limits. A study using a greater and more varied number of solutes and functionalities is required to determine whether such temperatures and compensation

TABLE 4
GROUP COMPENSATION RELATIONSHIPS ACCORDING TO EQN. 21

Solutes	Phase system	Slope coefficient ^a	n	r	Reference
2-,3-,4-substituted alkylbenzoates	Lichrosorb alkylsilicas/ methanol : water	-0.091	40	0.949	this study
3-,4-substituted alkylbenzoates	Lichrosorb alkylsilicas methanol : water	-0.077	28	0.934	this study
3-,4-substituted benzoic and phthalic acids	Hypersil SAS ^b alkylsilica/ acetonitrile : water + alkylbenzyltrimethylammonium pairing-ion	-0.075	10	0.920	Riley and Tomlinson, 1980
3-,4-substituted benzoic and phthalic acids	Hypersil SAS alkylsilica/ acetonitrile : water + 1,2-dien-Zn-(II) pairing-ion	-0.068	10	0.833	Riley and Tomlinson, 1980
3-,4-substituted benzoic acids	Spherisorb ^b alkylsilica/ acetonitrile : water + alkylbenzyltrimethylammonium pairing-ion	-0.068	10	0.976	Riley et al., 1979

^a Intercept values approximate to zero for all systems.

^b Hypersil SAS and Spherisorb are proprietary names.

lines are unique for all RP-HPLSC systems. The examination of the more modern alkyl-silica packing materials having little residual silanol character and per cent carbon loadings >10 , appears to be highly appropriate in this context.

Although the usefulness of the group compensation plot, (Eqn. 21), has been shown for comparing group retention in various phases, whether the coefficients of this equation, and others (Table 4), are unique for describing a hydrophobic phenomenon requires further information from, for example, liquid-liquid distribution studies before this can be established. Our current studies are directed towards this point. Meanwhile it is possible to examine only the thermodynamic behaviour of the much studied methylene group in other physicochemical equilibria. For transfer of this function from water to: (i) a non-polar organic liquid (Davis et al., 1974); and (ii) a pure solid phase (Somasundaran and Fuerstenau, 1972), $\Delta(\Delta G)$, $\Delta(\Delta H)$ and $\Delta(\Delta S)$ values are: (i) $-3.5 \text{ kJ} \cdot \text{mol}^{-1}$, $-1.4 \text{ kJ} \cdot \text{mol}^{-1}$ and $8 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$; and (ii) $-3.5 \text{ kJ} \cdot \text{mol}^{-1}$, $-7.4 \text{ kJ} \cdot \text{mol}^{-1}$ and $-12 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$, respectively. For the octyl/methanol : water (60 : 40) phase system, (Table 3) methylene group values of $-1.41 \text{ kJ} \cdot \text{mol}^{-1}$, $-2.53 \text{ kJ} \cdot \text{mol}^{-1}$ and $-3.62 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ are obtained. Although these values will be partly due to the presence of methanol, the sign of the values suggests that retention in RP-HPLSC is more akin to an adsorption rather than a partition process. We have commented on this point previously (Tomlinson et al., 1976).

A prime advantage of RP-HPLSC for providing indices describing drug distribution to a non-polar region is the greatly expanded range of values experimentally accessible using scaling relationships, such as given by Eqn. 4. Estimations using Eqns. 4-6 show that a correlated $\log K_d$ scale of -12 to $+12$ is possible using RP-HPLSC compared to a maximum range of -6 to $+6$ using shake-flask methods.

The use of modern liquid chromatography for providing information about the physicochemical properties of drugs is a useful alternative to conventional methodologies: whether the domain can be extended from describing hydrophobicity to other areas such as electron acceptor-donor equilibria and kinetic parameters is a future challenge.

We have reported briefly on this work previously (Tomlinson et al., 1978).

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REFERENCES

- Baker, J.K., Estimation of high pressure liquid chromatographic retention indices. *Anal. Chem.*, 51 (1979) 1693-1697.
- Baker, J.K., Rauls, D.O. and Borne, R.F., Correlation of biological activity and high-pressure liquid chromatographic retention index for a series of propranolol, barbiturate and anthranilic acid analogues. *J. Med. Chem.*, 22 (1979) 1301-1306.
- Berendsen, G.E. and de Galan, L., Role of the chain length of chemically bonded phases and the retention mechanism in reversed-phase liquid chromatography. *J. Chromatogr.*, 196 (1980) 21-38.
- Bush, I.E., Application of the R_M treatment in chromatographic analysis. *Meth. Biochem. Anal.*, 13 (1965) 357-438.
- Carlson, R.M., Carlson, R.E. and Klopperman, H.L., Determination of partition coefficients by liquid chromatography. *J. Chromatogr.*, 107 (1975) 219-223.

- Chen, B.-K. and Horváth, Cs., Evaluation of substituent contributions to chromatographic retention: quantitative structure-retention relationships. *J. Chromatogr.*, 171 (1979) 15-28.
- Collander, R., The partition of organic compound between higher alcohols and water. *Acta Chem. Scand.*, 5 (1951) 774-780.
- Conden, R., Gordon, A.H. and Martin, A.J.P., Qualitative analysis of proteins: a partition chromatographic method using paper. *Biochem. J.*, 38 (1944) 224-232.
- Davis, S.S., Higuchi, T. and Rytting, J.H., Determination of thermodynamics of functional groups in solutions of drug molecules. *Adv. Pharm. Sci.*, 4 (1974) 73-261.
- Franks, F., In F. Franks (Ed.), *Water: A Comprehensive Treatise*, Vol. 4, Academic Press, London, 1975, p. 243.
- Fujita, T., Iwasa, J. and Hansch, C., A new substituent constant, π , derived from partition coefficients. *J. Am. Chem. Soc.*, 86 (1964) 5175-5180.
- Henry, D., Block, J.H., Anderson, J.L. and Carlson, G.R., Use of high-pressure liquid chromatography for quantitative structure-activity relationship studies of sulphonamides and barbiturates, *J. Med. Chem.*, 19 (1976) 619-626.
- Horváth, Cs., Melander, W. and Molnár, I., Solvophobic interactions in liquid chromatography with nonpolar stationary phases. *J. Chromatogr.*, 125 (1976) 129-156.
- Huber, J.F.K., Meijers, C.A.M. and Hulsman, J.A.R.J., New method for prediction of partition coefficient in liquid-liquid systems and its experimental verification for steroids by static and chromatographic measurements. *Anal. Chem.*, 44 (1972) 111-116.
- Karch, K., Sebastian, I. and Halász, I., Preparation and properties of reversed phases. *J. Chromatogr.*, 122 (1976a) 3-16.
- Karch, K., Sebastian, I., Halász, I. and Engelhardt, E., Optimization of reversed-phase separations. *J. Chromatogr.*, 122 (1976b) 171-184.
- Karger, B.L., Gant, J.R., Hartkopf, A. and Weiner, P.H., Hydrophobic effects in reversed-phase liquid chromatography. *J. Chromatogr.*, 128 (1976) 65-78.
- Krug, R.R., Hunter, W.G. and Grieger, R.A., Enthalpy-entropy compensation. 1. Some fundamental statistical problems associated with the analysis of van 't Hoff and Arrhenius data. *J. Phys. Chem.*, 80 (1976a) 2335-2341.
- Krug, R.R., Hunter, W.G. and Grieger, R.A., Enthalpy-entropy compensation. 2. Separation of the chemical from the statistical effect. *J. Phys. Chem.*, 80 (1976b) 2341-2351.
- Leffler, J.E. and Grunwald, E. *Rates and Equilibria of Organic Reactions*, Wiley, New York, 1963, pp. 128-314.
- Löchl Müller, C.H. and Wilder, D.R., The sorption behaviour of alkyl bonded phases in reverse-phase, high performance liquid chromatography. *J. Chromatogr. Sci.*, 17 (1979) 574-579.
- Locke, D.C., Selectivity in reversed-phase liquid chromatography using chemically bonded stationary phases. *J. Chromatogr. Sci.*, 12 (1974) 433-437.
- Lumry, R. and Rajender, S., Enthalpy-entropy compensation phenomena in water solutions of proteins and small molecules: a ubiquitous property of water. *Biopolymers*, 9 (1970) 1125-1227.
- Martin, A.J.P., Some theoretical aspects of partition chromatography. *Biochem. Soc. Symp.*, Cambridge, England, 3 (1950) 4-20.
- McCall, J.M., Liquid-liquid partition coefficients by high-pressure liquid chromatography. *J. Med. Chem.*, 18 (1975) 549-552.
- Melander, W., Campbell, D.E. and Horváth, Cs., Enthalpy-entropy compensation in reversed-phase chromatography. *J. Chromatogr.*, 158 (1978) 215-225.
- Menheere, P., Devillez, C., Eon, C. and Guiochon, G., Method for prediction of partition coefficients in liquid-liquid chromatography. *Anal. Chem.*, 46 (1974) 1375-1378.
- Mirrlees, M.S., Moulton, S.J., Murphy, C.T. and Taylor, P.J., Direct measurement of octanol-water partition coefficients by high-pressure liquid chromatography. *J. Med. Chem.*, 19 (1976) 615-618.
- Miyake, K. and Terada, H., Preparation of a column with octanol-like properties for high-performance liquid chromatography. Direct measurements of partition coefficients in an octanol-water system. *J. Chromatogr.*, 157 (1978) 386-390.
- Molnár, I. and Horváth, Cs., Separation of amino-acids and peptides on non-polar stationary phases by high-performance liquid chromatography. *J. Chromatogr.*, 142 (1977) 623-640.

- Molnár, I. and Horváti, Cs., Catecholamines and related compounds. Effect of substituents on retention in reversed-phase chromatography. *J. Chromatogr.*, 145 (1978) 371–381.
- Nakae, A. and Muto, G., Chromatographic behaviour of alkylbenzenes and alkylbenzoates on porous micro-spherical poly(styrene-divinylbenzene) gel. *J. Chromatogr.*, 120 (1976) 47–54.
- Pierotti, G.J., Deal, C.H. and Derr, E.L., Activity coefficients and molecular structure. *Ind. Engng. Chem.*, 51 (1959) 95–102.
- Riley, C.M., Tomlinson, E. and Jefferies, T.M., Functional group behaviour in ion-pair reversed-phase high-performance liquid chromatography using surface-active pairing ions. *J. Chromatogr.*, 185 (1979) 197–224.
- Riley, C.M. and Tomlinson, E., Extrathermodynamic relationships in ion-pair HPLSC. *Proc. Anal. Div. Chem. Soc.*, 17 (1980) In press.
- Rittich, B., Polster, M. and Králik, O., Reversed-phase high-performance chromatography. I. Relationship between R_m values and Hansch's π parameter for a series of phenols. *J. Chromatogr.*, 197 (1980) 43–50.
- Scheraga, H.A., Interactions in aqueous solution. *Acc. Chem. Res.*, 12 (1979) 7–14.
- Sinanoğlu, O., Solvent effects in molecular associations. In B. Pullman (Ed.), *Molecular Associations in Biology*, Academic Press, 1968, pp. 427–445.
- Somasundaran, P. and Fuerstenau, D.W., Heat and entropy of adsorption and association of long-chain surfactants at the alumina–aqueous solution interface. *Trans. Soc. Mining Engng.*, 252 (1972) 275–279.
- Tanaka, N. and Thornton, E.R., Structural and isotopic effects in hydrophobic binding measured by high-pressure liquid chromatography. A stable and highly precise model for hydrophobic interactions in biomembranes. *J. Am. Chem. Soc.*, 99 (1977) 7300–7307.
- Tjaden, U.R., Ph.D. Thesis, 1976, University of Amsterdam.
- Tjaden, U.R., Kraak, J.C. and Huber, J.F.K., Rapid trace analysis of barbiturates in blood and saliva by high-pressure liquid chromatography. *J. Chromatogr.*, 143 (1977) 183–194.
- Tomlinson, E., Chromatographic hydrophobic parameters in correlation analysis of structure–activity relationships. *J. Chromatogr.*, 113 (1975) 1–45.
- Tomlinson, E., Poppe, H. and Kraak, J.C., Extrathermodynamic group contribution values determined by high pressure reversed-phase liquid/solid chromatography. *J. Pharm. Pharmacol., Suppl.*, 28 (1976) 43P.
- Tomlinson, E., Poppe, H. and Kraak, J.C., A group contribution approach to the HPLC analysis of drugs. In E. Reid (Ed.), *Blood Drugs and Other Analytical Challenges*, (Vol. 7 of *Methodological Surveys in Biochemistry*), Ellis Horwood, Chichester 1978, pp. 207–212.
- Tomlinson, E., Davis, S.S. and Mukhayer, G.I., Ionic interaction and phase stability. In K.L. Mittal (Ed.), *Solution Chemistry of Surfactants*, Vol. 1, Plenum, New York, 1979, pp. 3–43.
- Tomlinson, E. and Davis, S.S., Interactions between large organic ions of opposite and unequal charge. III. Enthalpy–entropy linear compensation and application of solvophobic theory. *J. Colloid Interface Sci.*, 76 (1980) 563–572.
- Twitchett, P.J. and Moffat, A.C., High-pressure liquid chromatography of drugs. An evaluation of an octadecylsilane stationary phase. *J. Chromatogr.*, 111 (1975) 149–157.
- Unger, S.H., Cook, J.R. and Hollenberg, J.S., Simple procedure for determining octanol–aqueous partition, distribution, and ionization coefficients by reverse-phase high-pressure liquid chromatography. *J. Pharm. Sci.*, 67 (1978) 1364–1367.
- Unger, S.H. and Feuerman, T.F., Octanol–aqueous partition, distribution and ionization coefficients of lipophilic acids and their anions by reversed-phase high-performance liquid chromatography. *J. Chromatogr.*, 176 (1979) 426–429.
- Veith, G.D., Austin, N.M. and Morris, R.T., A rapid method for estimating log P for organic chemicals. *Water Res.*, 13 (1979) 43–47.
- Vigh, Gy. and Varga-Puchony, Z., Influence of temperature on the retention behaviour of members of homologous series in reversed-phase high-performance liquid chromatography. *J. Chromatogr.*, 196 (1980) 1–10.
- Yamana, T., Tsuji, A., Miyamoto, E. and Kubo, O., Novel method for determination of partition coefficients of penicillins and cephalosporins by high pressure liquid chromatography. *J. Pharm. Sci.*, 66 (1977) 747–749.