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THE POSITION OF REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY AMONGST SOLVENT PARTITIONING TECHNIQUES

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1. INTRODUCTION

In the same way as the dissociation of acids, a partition coefficient P can be conveniently defined as an equilibrium constant:

$$P = k_a/k_b \tag{1}$$

where k_a and k_b represent the rates of transfer of a solute from one partner of the solvent system to the other. A consistent extension of this parallel implies that in addition to Hammett's equation:

$$\log K_s/K_0 = \varrho\sigma \tag{2}$$

which describes the effect of substitution on a K_0 valid for the unsubstituted structure[1,2], there must be some sort of analogue expressing the effect of a substitution on the partition coefficient of the unsubstituted structure:

$$\log P_s/P_0 = \varrho'\pi \tag{3}$$

In eqn. 2, K_s and K_0 represent the equilibrium constant for the reactions of substituted and unsubstituted compounds, respectively; σ is an electronic constant that depends on the nature and position of the substituent and ϱ is a constant associated with a given type of reaction and the conditions under which it takes place. In eqn. 3, P_s and P_0 represent the partition coefficients of substituted and unsubstituted molecules, respectively, in systems consisting of two solvents (one is water) that are immiscible or partially immiscible; π is the analogue of σ in eqn. 2 and denotes the

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transfer free-energy change of the substituent; ϱ' is the analogue of ϱ in eqn. 2 and denotes a constant dependent on the nature of the solvent system employed in the measurements of the two partition values.

Hammett performed a standardization of eqn. 2 with the dissociation of benzoic acid in water at 25°C, for which ϱ was taken to be 1.000. Eqn. 2 can thus be written as

$$\log K_s/K_0 = \sigma \tag{4}$$

The definition of Hansch and co-workers[3–5] for the hydrophobic substituent constant (the contribution of a substituent replacing a hydrogen atom to the lipophilicity of a parent structure as expressed in eqn. 5) will mean that by analogy with the Hammett standardization performed in eqn. 4, the constant ϱ' in eqn. 3 should be fixed at 1.000 for a solvent pair to be selected as the standard partition system:

$$\log P_s/P_0 = \pi \tag{5}$$

For several reasons, Hansch chose the octanol–water system[6].

The magnitude of the constant ϱ provides important information when Hammett's equation is applied in reaction kinetic studies, and ϱ can then be taken as a measure of the susceptibility of the reaction under study to electronic influences from the substituent. Within this context, the algebraic sign of ϱ is of great importance, it being positive if the reaction is favoured by a low electron density and negative if the reaction is favoured by a high electron density at the reaction centre.

By analogy, the significance of the constant ϱ' in eqn. 3 should be emphasized; this ϱ' will serve as a measure of the susceptibility of the chosen partition system to the lipophilic behavioural pattern incorporated in the substituent, with a potentially major role of the algebraic sign of ϱ'.

2. SOLVENT PARTITION; COLLANDER-TYPE EQUATIONS

The octanol–water system is preferred to any other system in most partition studies[6,7], especially those performed in connection with work in pharmacochemistry.

The following systems form a descending series as regards frequency of application: octanol–water, chloroform–water, cyclohexane–water, diethyl ether–water, triglyceride–water, *n*-heptane–water, benzene–water, oleyl alcohol–water, isobutanol–water, carbon tetrachloride–water and, finally, about some sixty others which are only occasionally used[8].

Smith[9] was the first to suggest that in principle the partition coefficients measured in different solvent systems could be linked up with each other. Relationships of this type were worked out in practice by Collander[10,11].

2.1. Collander equations

In its generalized form, the Collander equation is as follows:

$$\log P_a = \varrho \log P_b + q \tag{6}$$

where P_a and P_b are partition coefficients and ϱ and q are constants that are characteristic of the two solvent systems employed.

Collander established that the fit implied in eqn. 6 became poorer as the polarity differences between the organic solvents in the two partition systems became larger. Thus a good fit can be expected for the octanol–water *versus* the pentanol–water system and for the *n*-heptane–water *versus* the cyclohexane–water system, while the combination of the cyclohexane–water *versus* the octanol–water system is typical of a poorly fitting eqn. 6.

Collander argued that the effects observed resulted from differences in hydrogen bonding, more of these bonds being formed between solute and solvent molecules in one case than in the other. What the slopes and intercepts of the straight lines as represented by eqn. 6 really signify remains uncertain, however.

2.2. *Leo–Hansch–Elkins equations*

Hansch[12] and Leo and co-workers[13,14] have been actively engaged in the extension of the Collander equation to a wide variety of partitioning solvent systems. It appeared that for the equation to be applied correctly to fit solvent systems with widely divergent polarities of the organic solvent components, the partitioned solutes need to be divided over two regression equations, one of them being related to H-donor and the other to H-acceptor solutes. An example is the regression diethyl ether–water *versus* octanol–water. In all, 103 data points were qualified for this evaluation. This led to the following Collander equation:

$$\log P_{\text{ether–w}} = 1.186 \log P_{\text{oct–w}} - 0.472 \quad (7)$$
$$n = 103;\ r = 0.929;\ s = 0.477;\ F = 640;\ t = 25.3$$

(where the subscript w represents water), the donor–acceptor separation of these 103 data points resulting in the following two equations:

$$\log P_{\text{ether–w}} = 1.133 \log P_{\text{oct–w}} - 0.168 \quad (8)$$
$$n = 71 \text{ donors};\ r = 0.988;\ s = 0.185;\ F = 2829;\ t = 53.2$$

$$\log P_{\text{ether–w}} = 1.141 \log P_{\text{oct–w}} - 1.068 \quad (9)$$
$$n = 32 \text{ acceptors};\ r = 0.956;\ s = 0.328;\ F = 320;\ t = 17.9$$

The statistics of the last two equations, especially those of the donors, show considerable improvement in all respects. Leo and co-workers have not always been so successful in their attempts to obtain fully acceptable regression equations through this donor–acceptor separation. Sometimes it was necessary to set up a class of neutrals in addition to donors and acceptors. This is the case, for example, with the chloroform–water *versus* octanol–water system and the carbon tetrachloride–water *versus* octanol–water regressions.

Where polarity differences are very large, as with the cyclohexane–water *versus* the octanol–water system, it is still possible to obtain a reasonable fit for acceptor structures but no longer for donor structures ($r = 0.761$). Leo and co-workers in their comments on the slopes and intercepts of the proposed regression equations, postu-

lated that if hydrogen bonding were accounted for correctly, the slopes of all solvent regression equations would be unity. The intercept values of the solvent equations they considered to be clearly related to the extent to which water is dissolved in the organic phase of the solvent system. The following equation would indicate this:

$$\log [H_2O] = 1.076\, I + 0.249 \qquad (10)$$
$$n = 17;\ r = 0.978;\ s = 0.246;\ F = 340;\ t = 18.4$$

where $[H_2O]$ is the water concentration at saturation level in the organic phase of the solvent system and I is the intercept values from seven "donor" and ten "sole" equations*.

2.3. Rekker equation

The approach of Leo and co-workers to solvent regression as outlined above may be defined as a differentiation in the solute group and that of Rekker[8] as a differentiation in the solute structure.

The hydrophobic fragmental constant f permits the transformation of the Collander equation (eqn. 6) into

$$\Sigma f_a = \varrho \Sigma f_b + q \qquad (11)$$

where Σf_a represents the summation of a series of fragmental constants valid for solvent system a and Σf_b the summation of the same series in solvent system b.

There is a distinct difference between this hydrophobic fragmental constant f and Hansch's hydrophobic substituent constant π (eqn. 5), the former being defined as the contribution of a constituent part of a structure to total lipophilicity, which can thus be expressed as

$$\log P = \sum_{1}^{n} a_n f_n \qquad (12)$$

where f denotes the hydrophobic fragmental constant, a a numerical factor indicating the incidence of a given fragment in the structure and n the number of fragments.

The advantage of eqn. 11 over the Collander equation and those of Leo and co-workers is that it admits differentiation in the Σf terms; the equation can thus be easily transformed into

$$\Sigma f_1(a) + \Sigma f_2(a) = \varrho[\Sigma f_1(b) + \Sigma f_2(b)] + q \qquad (13)$$

where the subscripts 1 and 2 indicate non-polar and polar fragments, respectively, and an even further differentiation would result in

* By a "sole" equation is meant a solvent equation that ensures a proper fit for all data points without the necessity for donor–acceptor separation. Sole equations are the rule where no large polarity differences occur among the solvent systems compared; a case in point is the butanol–water *versus* octanol–water regression.

$$\Sigma f_1(a) + \Sigma f_2(a) = \varrho'\Sigma f_1(b) + \varrho''\Sigma f_2(b) + q' \quad (14)$$

In eqn. 14 different slopes, if relevant, are allowed for non-polar and polar fragments, accompanied by a possible change of the intercept q into q'.

A study with twelve different solvent systems *versus* the octanol–water system[8] clarified the essentials of eqn. 14 and, consequently, it may be concluded that:

(1) The separation of non-polar from polar fragments as implied in eqn. 14 is very easy to attain in practice, it now being definitely established for each system whether a given fragment is non-polar (usually hydrophobic) or polar (usually hydrophilic).

(2) Lipophilic structures solely constructed from lipophilic fragments can invariably be treated with an interceptless equation:

$$\Sigma f_1(a) = \varrho'\Sigma f_1(b) \quad (15)$$

The slope in eqn. 15 does not represent unity, but seems to vary with the mutual solubility of water and the chosen organic partner in the partition system.

(3) The apparent complexity of eqn. 14 resulting from the two mutually differing slopes ϱ' and ϱ'' for hydrophobic and hydrophilic fragments, respectively, can be easily disregarded by means of the following equation:

$$\varrho''\Sigma f_2(b) = \varrho'\Sigma f_2(b) + (c_M)_a\Sigma kn \quad (16)$$

by which, when we simultaneously equate the slope values ϱ' and ϱ'', the intercept q' of eqn. 14 will attain the value:

$$q' = (c_M)_a\Sigma kn \quad (17)$$

where $(c_M)_a$ is a constant factor for solvent system a and may be written as:

$$(c_M)_a = \varrho'(c_M)_{oct} = 0.289\ (\pm\ 0.002)\varrho' \quad (18)$$

The last expression implies that the anomalous behaviour of hydrophilic fragments can be quantified in portions of 0.289 ϱ'. Their incidence (see eqns. 16 and 17) is rendered by Σkn, where kn represents a "key number" and the symbol Σ indicates that several hydrophilic groups in one structure contribute their own 0.289 ϱ' incidence to the overall partitional behaviour of the entire structure.

When again log P notations are used, the equation for conversion of a given solvent system in the octanol–water standard system can now be written as

$$\log P_a = \varrho \log P_{oct} \mp (c_M)_{oct}\varrho\Sigma kn \quad (19)$$

This equation permits the following differentiations: (a) solvent systems with $\varrho = 1$, which will appear to be accompanied by $kn = 0$; this solvent system type is indicated as *iso*-discriminative; (b) solvent systems with $\varrho > 1$, which will appear to be coupled to the operation of the upper algebraic sign; this solvent system type is indicated as *hyper*-discriminative; (c) solvent systems with $\varrho < 1$, which will appear to be coupled

to the operation of the lower algebraic sign; this solvent system type is indicated as *hypo*-discriminative.

3. THIN-LAYER CHROMATOGRAPHY APPLYING REVERSED-PHASE SYSTEMS

Since Tomlinson[15] dealt with various practical and fundamental aspects of thin-layer chromatography in an excellent review, a few brief sections directly concerned with the question which we are attempting to answer may suffice here. This question is, what exactly is the position occupied by reversed-phase thin-layer chromatography (RPTLC) in the complexity of available solvent partitioning techniques?

By analogy with eqn. 5, an equation can be designed for RPTLC and its variants to express the effect of substitution of a hydrogen atom by another atom (or group) on the transport across a thin-layer plate surface or through a chromatographic column:

$$\Delta R_M(\mathrm{X}) = R_M(\mathrm{X}) - R_M(\mathrm{H}) \tag{20}$$

where R_M represents a term proposed by Bate-Smith and Westall[16] and connected as follows with the directly experimentally obtainable chromatographic R_F values:

$$R_M = \log (1/R_F - 1) \tag{21}$$

Although the theories underlying paper and thin-layer chromatography[17,18] were derived for systems in which partition processes play an exclusive role, many authors are prepared to extend them to cases where adsorption is intimately involved. Where and to what extent a major contribution of adsorption has affected the results obtained is either not or insufficiently recorded.

Reversed-phase variants of TLC are, as we know from own experience, far less hazardous for the investigator in that he will not have uninterpretable sets of R_M values in his hands. Also, we have available the simple graphic criterion proposed by Hulshoff and Perrin[19] to establish whether an experiment is solely ruled by partitional behaviour or not.

Although partition coefficients may be regarded as genuine equilibrium constants and R_M values as being derived from steady-state functions and not from true equilibrium situations, the Collander equation as discussed above proves applicable to either systems where two R_M sets are interconnected or to systems where solvent partition data are coupled to R_M values. A few examples of this type of regression equation are given below.

(a) Penicillins —5% silicone oil[20].

$$R_M = 0.434 \log P - 0.225 \tag{22}$$
$$n = 6;\ r = 0.892;\ s = 0.236$$

(b) Cinnamic acid derivatives —non-reversed phase, benzene–formamide impregnated paper[21].

$$\log P = 1.715\ R_M - 1.747 \tag{23}$$
$$n = 35;\ r = 0.984;\ s = 0.192$$

(c) Testosterone esters —5% silicone oil, acetone–water (54:46)[22].

$$R_M = 0.288\,\pi - 0.143 \quad (24)$$
$$n = 14;\ r = 0.964;\ s = 0.119$$

(d) Acetanilides and triazinones —polyamide[23].

$$\Delta R_M = 0.456\,\pi + 0.027 \quad (25)$$
$$n = 42;\ r = 0.991;\ s = 0.075$$

(e) Heterocyclic ring-substituted sulphonamides —silicone oil[24].

$$\pi = 0.993\,R_M + 0.485 \quad (26)$$
$$n = 16;\ r = 0.961;\ s = 0.191$$

(f) Thiolactams[25].

$$R_M(\text{butanol–acetone–water}) = 1.604\,R_M(\text{dioxane}) - 0.606 \quad (27)$$
$$n = 5;\ r = 0.966;\ s = 0.041$$

These equations will be discussed in more detail in the following section.

4. A PROPOSED R_M FRAGMENTATION PROCEDURE

One of the objections to the π system[8] is that it is very unwieldy with complicated structures. Further, there is the risk that one is tempted to perform the calculations with the following equation:

$$\log P = \sum_1^n \pi_n \quad (28)$$

TABLE 1

COMPARISON OF f WITH π VALUES

al means aliphatic fragment attachment; ar means aromatic fragment attachment.

Fragment	*f*	π	Δ	*Fragment*	*f*	π	Δ
CH_3	0.701	0.50	0.20	OH (al)	−1.470	−1.16	−0.31
CH_2	0.519	0.50		OH (ar)	−0.314	−0.67	0.36
CH	0.337	0.50*		Cl (al)	0.057	0.39	−0.33
C_6H_5	1.840	2.13	0.29	Cl (ar)	0.924	0.71	0.21
C_6H_4	1.658	2.13		NO_2 (al)	−0.920	−0.85	−0.07
C_6H_3	1.476	2.13		NO_2 (ar)	−0.053	−0.28	0.23
H	0.182	0.00	0.18	NH_2 (al)	−1.420	−1.19	−0.23
				NH_2 (ar)	−0.842	−1.23	0.39
				$NHCH_3$ (al)	−1.113**	−0.67	−0.44
				$NHCH_3$ (ar)	−0.246**	−0.47	0.22
				$N(CH_3)_2$ (al)	−0.683**	−0.32	−0.36
				$N(CH_3)_2$ (ar)	0.473**	0.18	0.29
				$CONH_2$ (al)	−1.975	−1.71	−0.27
				$CONH_2$ (ar)	−1.108	−1.49	0.37

* Requires application of a branching correction.

** By addition from f values of the constituent parts of the fragment.

This equation implies that a partition value can be construed by simply adding together the π contributions of the constituent groups[26] without realizing, however, that eqn. 28 is an incorrect transformation of the original Hansch equation (eqn. 5).

Not only the objection just mentioned but also others can be entirely met by changing to the hydrophobic fragmental constant f. In all, for about 100 of these fragmental constants, reliable values for use in the octanol–water system have been made available[27] and they will cover almost the entire field of structures of interest to the pharmacochemist.

Table 1 gives a few examples of f values compared with their π values and emphasizes some of the marked differences between the f and the π systems.

As soon as one extends the parallel from log P to R_M by defining ΔR_M analogously to π (*cf.*, eqns, 5 and 20), the objections we raised against the π system will be undiminishedly valid for the ΔR_M system. Also, it follows as a logical consequence that one has to perform an adequate R_M fragmentation procedure that replaces the ΔR_M substituent concept given in eqn. 20 by the following:

$$R_M = \sum_1^n a_n \tau_n \tag{29}$$

where τ represents the fragmental R_M contribution of a constituent part of a structure to its total R_M value and a and n are as in eqn. 12.

Eqn. 29 will appear substantially to meet the objections[15] inherent in the "ΔR_M" equivalent of eqn. 28:

$$R_M = \Sigma \Delta R_M \tag{30}$$

A thorough consultation of the literature shows that the concept underlying eqn. 29 is not entirely new. In 1949 Martin[28] proposed a treatment of R_M data by means of the following equation:

$$R_{MB} = R_{MA} + R_{MX} + R_{MY} + R_{MZ} \tag{31}$$

which implies that the R_M parameter of a given structure (B) is made up by the R_M values of its components (A, X, Y and Z); hence eqn. 31 is not really different from eqn. 29.

The principle has been incidentally applied in one or another form. Pardee[29] applied a partial fragmentation in order to establish a relationship between the R_F values obtained on paper chromatograms for peptides and the amino acids of which they are composed. From the investigations published later, we select the one of Marcinkiewicz and co-workers[30], performed with phenols and a number of derivatives and related structures on paper impregnated with ethyl oleate using 25% aqueous ethanol as the mobile phase. Their calculation of the R_M value of a compound makes use of appropriate R_M fragments (denoted group and atomic R_M parameters) derived from a suitable set of structures; in this respect the method resembles our approach. On the other hand, however, Marcinkiewicz and co-workers applied corrections that are apparently more in line with the procedure followed in the calculation of molecular refractivities: ring attachments, double bonds, etc.

The principle aim of what we propose is to apply to R_M a fragmentation procedure in line with our log P fragmentation, implying an optimal application of

equations of the Collander type, thus creating the possibility of qualifying the position of reversed-phase thin-layer chromatography amongst the several solvent partition techniques.

The basic incorrectness of eqns. 28 and 30 is connected with the fact that the concept *substituent* constant implies the occurrence of the parameters log P and R_M with equal frequency in the left- and right-hand sides of these equations. However, the right-hand side of eqn. 28 contains no log P and in that of eqn. 30 R_M is absent. Eqns. 28 and 30 can be corrected adequately by

$$f(\mathrm{X}) - \pi(\mathrm{X}) = f(\mathrm{H}) \tag{32}$$

and

$$\tau(\mathrm{X}) - \Delta R_M(\mathrm{X}) = \tau(\mathrm{H}) \tag{33}$$

respectively, and transformed to sets of usable equations. For aliphatic structures, these are as follows:

$$\log P = \sum_{k+l} \pi + 0.182\,(k + l - m)$$

$$R_M = \sum_{k+l} \Delta R_M + \tau(\mathrm{H})\,(k + l - m) \tag{34}$$

where k is the number of functional groups in the considered structure, l is the number of CH_3 and/or CH_2 groups, m is the number of branchings on carbon atoms and 0.182 represents the f value of the H atom.

Similar transformations can be achieved for aromatic structures and for structures of a mixed aliphatic–aromatic type.

The practical use of this sort of equation requires the availability of correct π and ΔR_M sets, respectively, and, as can be seen in Table 1, this is by no means attained for the π system. In particular this is true for the aliphatic πs; it is our feeling that for their calculations too frequent use has been made of eqn. 28, resulting in a system on an unstable basis.

The aliphatic π system derives its basic value $\pi(CH_3) = \pi(CH_2)$ from the log P measurement of pentane[31,34], *i.e.*, $1/5 \cdot 2.50 = 0.50$, and the other π values are then obtained by a simple subtraction of $(n + 1) \cdot 0.50$ from the log P values of the structures $CH_3(CH_2)$–X. An example is $\pi(OH)$ from log $P(CH_3OH) - 1 \cdot 0.50 = -0.66 - 0.50 = -1.16$ or from log $P(CH_3CH_2CH_2OH) - 3 \cdot 0.50 = 0.34 - 1.50 = -1.16$.

More accurate measurements of the octanol–water partition for pentane afforded the values 3.23 and 3.39[32], however, which entail a distinctly higher $\pi(CH_3)$ value: $1/5 \cdot 3.32 = 0.66$. In turn, a value of 0.66 would yield $\pi(OH)$ values of -1.32 and -1.64, respectively, depending on whether one starts from log P (CH_3OH) or from log $P(CH_3CH_2CH_2OH)$, in other words, the original consistency between the two log P data of methanol and propanol is eliminated completely by changing to a seemingly more correct $\pi(CH_3)$ value.

The rejection of eqns. 28 and 30 implies that the correctness of the Collander-type equations (eqns. 22–27), referred to in the preceding section, need to be checked more closely.

It then appears that eqns. 22, 23 and 27 are correct because the left- and right-hand sides contain one factor describing overall lipophilicity (log P or R_M), that eqn. 25 is correct because neither the left- nor the right-hand side contain such a factor, and that eqns. 24 and 26 are incorrect because in the former the right-hand side and in the latter the left-hand side is without this factor.

It will be clear that by analogy with eqn. 19 that any equation relating TLC data to solvent partition values must have the following general form:

$$R_M = \varrho \log P_{oct} \mp (c_M)_{oct} \varrho \Sigma kn \tag{35}$$

where, if necessary, one can choose a solvent pair other than octanol–water. The second term of the right-hand side can be simply written as Δh because it results from hydration differences, and log P can, if required, be replaced by an f-summation term:

$$R_M = \varrho \sum_1^n a_n f_n \mp \Delta h \tag{36}$$

While searching for a reliable set of TLC data for a further test of the merits of eqn. 36, we encountered the almost classical paper by Boyce and Milborrow[33].

5. A RE-EVALUATION OF THE EXPERIMENTAL DATA OF BOYCE AND MILBORROW[33]

Boyce and Milborrow examined eight N-*n*-alkyltriphenylmethylamines by a reversed-phase TLC method, applying 5% liquid paraffin on silica gel and acetone–water compositions ranging from 0.9 to 0.5 for elution.

Table 2 lists their R_M values for the acetone–water composition 0.7, which can be correlated with the f values of the alkyl groups (Σf) as follows:

$$R_M = 0.245(\pm 0.015)\, \Sigma f - 0.442(\pm 0.042) \tag{37}$$
$$n = 8;\ r = 0.998;\ s = 0.021;\ F = 1557$$

TABLE 2

REVERSED-PHASE THIN-LAYER CHROMATOGRAPHIC BEHAVIOUR OF $\varnothing_3 \equiv C\text{–}NH\text{–}R$

Liquid paraffin on SiO_2/acetone–water (0.75). Figures given in round brackets behind eluent compositions indicate the organic eluent component (acetone) as fraction of the total.

R	R_M	Σf	R_M (clc)*
CH_3	−0.305	0.701	−0.271
C_2H_5	−0.119	1.220	−0.143
C_3H_7	0.008	1.739	−0.016
C_4H_9	0.115	2.258	0.111
C_5H_{11}	0.225	2.777	0.238
C_6H_{13}	0.368	3.296	0.365
C_7H_{15}	0.485	3.815	0.493
C_8H_{17}	0.620	4.335	0.620

* Estimate values obtained from eqn. 37.

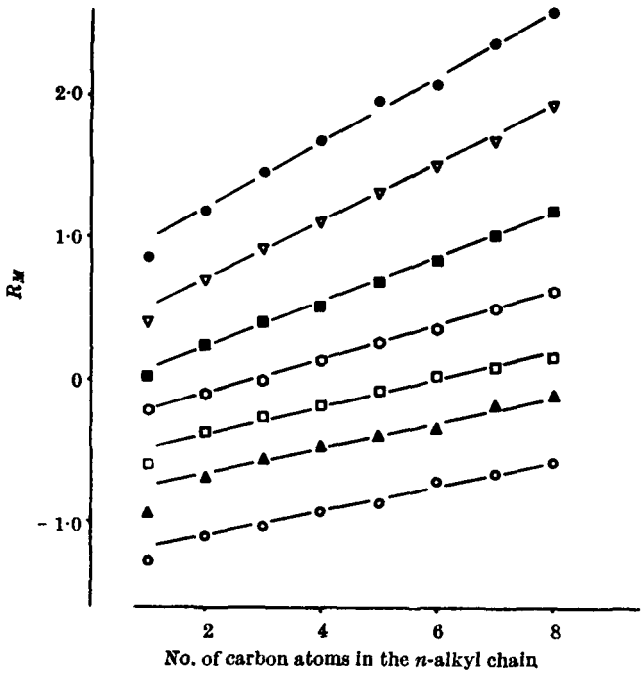

Fig. 1. Relationship between R_M and number of carbon atoms in the alkyl chain of N-*n*-alkyltritylamines obtained with varying acetone–water elution ratios. Acetone fraction in the mobile phase: ●, 0.50; ▽, 0.56; ■, 0.67; ⬡, 0.75; □, 0.82; ▲, 0.83; ○, 0.91. (Reprinted by kind permission from *Nature (London)*, 208 (1965) 538; Copyright 1965, Macmillan Journals Ltd.).

If log P (to be obtained by *full f* summation) had been used as an independent parameter, the result would have been

$$R_M = 0.245 \log P_{oct} - 1.388 \tag{38}$$

(It should be noted that eqn. 38 can be obtained by a direct transformation from eqn. 37 without the necessity of performing another regression.)

The ϱ value of 0.245 would label the RPTLC system employed as *hypo*-discriminative towards the solvent partition system octanol–water, whereas the negative value of Δh (-1.388) indicates that in the TLC experiment the hydratable group in the structure (here the tertiary N) has definitely gained in hydrophilicity, in other words, traverses the thin-layer plate in a relatively more hydrated form.

These statements seem to conflict with what we usually find for solvent partition regressions, where *hypo*-discrimination in the ϱ value is associated with a positive intercept value; they indicate that the RPTLC procedure is dualistic with regard to the octanol–water partition system; it responds with *hypo*-discrimination towards lipophilic fragments, but towards hydratable (hydrophilic) fragments it behaves reversely, necessitating the appearance of a negative algebraic sign in the second right-hand term of eqn. 38.

For the purpose of a more detailed study, the RPTLC experiments by Boyce

TABLE 3

TRANSFORMATION OF R_M–LIPOPHILICITY REGRESSIONS

*ac/w** *(exp.)*	$R_M = an + b$		*ac/w** *(calc.)*	$R_M = \varrho\Sigma f + q$		$\Sigma\tau_M$ $\varnothing_3C + NH$	Δh
	a	b		ϱ	q		
0.50	0.219	0.768	0.48	0.422	0.691	1.629	−0.938
0.56	0.197	0.300	0.57	0.380	0.231	1.467	−1.236
0.67	0.154	−0.072	0.67	0.297	−0.126	1.147	−1.276
0.75	0.114	−0.312	0.74	0.220	−0.352	0.849	−1.201
0.82	0.094	−0.570	0.80	0.181	−0.603	0.699	−1.302
0.83	0.085	−0.828	0.85	0.164	−0.858	0.633	−1.491
0.91	0.083	−1.260	0.91	0.160	−1.289	0.618	−1.907

* ac/w = amount of acetone (v/v) as a fraction of the total.

and Milborrow with variable acetone–water elutions prove extremely instructive. Their paper does not list the original chromatographic data in detail but through a suitable photographic enlargement of their graph as reproduced in Fig. 1, all of the desired information became available with sufficient accuracy.

Columns 2 and 3 in Table 3 show the seven equations represented by the straight lines in Fig. 1. They are of the type

$$R_M = an + b \tag{39}$$

where n is the number of carbon atoms in the alkyl chain of the investigated compounds. By means of

$$\varrho = a/f(CH_2)$$

and

$$q = a + b - \varrho \cdot f(CH_3)$$

eqns. 39 can be transformed into equations of the type

$$R_M = \varrho\Sigma f + q \tag{40}$$

Columns 5 and 6 in Table 3 give detailed information on the equation-set 40.

The symbol Σf in eqn. 40 represents the sum of the hydrophobic fragmental constants of the variable substituent of the tritylamine. When equations of type 40 instead of type 39 or equations parameterized in π are used, it will be seen that several so-called "first member anomalies" no longer occur, because neglect of the proper contribution of H to the overall lipophilicity and an equation of CH_3 and CH_2 is ruled out (see Table 4).

The question of how successful the manipulations with Fig. 1, resulting via eqns. 39 in eqns. 40, really have been can be answered via eqn. 41, which was derived from another graph of Boyce and Milborrow and reflects the relationship between the

TABLE 4

ORIGIN OF SOME "FIRST MEMBER ANOMALIES" IN PARTITION AND REVERSED-PHASE THIN-LAYER EXPERIMENTS

System applied for calculation	*Substituent*				
	*H (norm.)**	*H (neg.)***	CH_3	C_2H_5	C_3H_7
n	0	n.r.***	1	2	3
π	0.00	n.r.	0.50	1.00	1.50
f	0.182	0.471	0.701	1.220	1.739

* H (norm.) = H attached to aliphatic or aromatic C atoms as far as they do not participate in a functional group.

** H (neg.) = H attached to an electronegative centre such as C=O, COOH, R–CONH–.

*** n.r. = not recognized.

R_M values of the N-hexyl derivative and the relevant ac/w ratios employed in the RPTLC experiments:

$$R_M = -6.52 \text{ ac/w} + 5.20 \tag{41}$$

Substitution of 3.296 for Σf (= hexyl value) in the seven eqns. 40 yields seven R_M values, which, in turn, afford the seven ac/w ratios given in column 4 of Table 3. Obviously, they agree very well with those in column 1.

With ϱ known, it is possible to perform an "eqn. 37–38" type transformation for the seven regression eqns. 40; starting from $f(\varnothing_3C + NH) = 3.861$ one obtains the $\Sigma\tau(\varnothing_3C + NH)$ values as given in column 7 through multiplication by ϱ, and by subtracting these last values from q the values for Δh (column 8) are established.

We observe a remarkable coherence between the values of ϱ, Δh and the ac/w

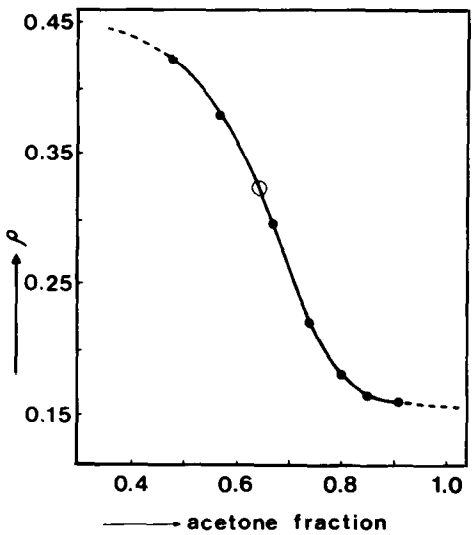

Fig. 2. Plot of ϱ *versus* the composition of the acetone–water eluate (acetone fraction).

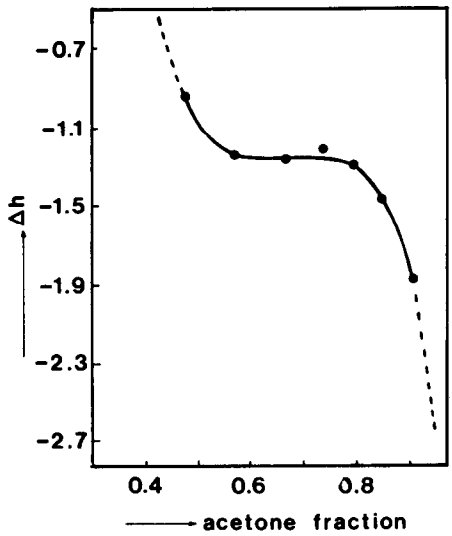

Fig. 3. Plot of Δh *versus* the composition of the acetone–water eluate (acetone fraction).

ratios used for elution, as expressed graphically in Figs. 2 and 3, respectively.

The ϱ–ac/w plot in Fig. 2 suggests a sigmoid shape with limiting ϱ values of approximately 0.50 and 0.15.

It is difficult to establish the extent to which experimental imperfectness is responsible for the apparent sigmoid form of the graph and if not the top extreme R_M values represented graphically by the top and bottom lines of Fig. 1 (R_Ms larger than 0.75 or smaller than -0.85, corresponding with R_F s smaller than 0.15 or larger than 0.88) should be considered suspect. In this respect we would refer to comparable observations by D'Amboise and Hanai[35] with acetonitrile–water elution in a series of high-performance liquid chromatographic experiments. Their findings indicate that at extreme concentrations (acetonitrile concentrations higher than 80% and lower than 20%) the mechanism of retention is more complex than a pure hydrophobic effect. They believe that hydrogen bonding competes with hydrophobic effects at high acetonitrile content whereas coating of the packing material with water molecules could be responsible for the behaviour at low acetonitrile content. Reference was made to similar observations by Horváth *et al.*[36].

The inflection point in Fig. 2, where the sensitivity of ϱ to ac/w fluctuations is greatest, can easily be located with the help of Fig. 3, because the Δh values appear to be remarkably constant over a fairly wide range of ac/w ratios: $\Delta h = -1.253$ (with s.d. 0.044) over an ac/w ratio varying from 0.55 to 0.80. With this in mind, the location of the inflection point in Fig. 2 can be established easily. As at this point the $R_M/\Sigma f$ equation is interceptless:

$$R_M = \varrho \Sigma f \tag{42}$$

we can write

$$\Sigma\tau(\varnothing_3C + NH) + \Delta h = 0$$

and

$$\Sigma\tau(\varnothing_3C + NH) = 1.253$$

and because $\Sigma f(\varnothing_3C + NH) = 3.861$, the value of ϱ in the inflection point of Fig. 2 must be $1.253/3.861 = 0.325$, which will enable us to read an ac/w value of 0.64 from the sigmoid curve.

6. DISCUSSION

To begin with, the RPTLC process usually reflects a partition experiment in the mobile phase–stationary phase system. In the experiments performed by Boyce and Milborrow the system actually consisted of acetone–water as the one partner and paraffin oil as the other, and this makes the situation essentially more complex than normally is encountered in a shaking-flask procedure making use of a common solvent pair.

The presence of acetone will partially abolish the immiscibility of the paraffin oil–water system, shifting partitions in favour of the aqueous solvent partner. This implies a decrease in the R_M values and an enhancement of the negative Δh term. The extremes of the range of possibilities can be described as follows: (a) acetone is (almost) entirely lacking —the RPTLC system has its maximal discrimination in combination with a virtually immobile eluent front*; (b) water is (almost) entirely lacking— the RPTLC system has its minimal discrimination in combination with an optimally mobile eluent front.

The plate equivalent of the hydrophobic fragmental constant of the secondary NH group, the τ(NH) factor, is singularly constant over a large range within the above indicated extremes. It is difficult to indicate how far this claim can be extended in the limit situations described under (a) and (b), the curves in Figs. 2 and 3 suggesting that irregularities cannot be avoided.

In illustration of this, some fragment calculations are presented in an elaborated form in Table 5, from which it appears that τ(NH) has a value of -1.74 (s.d. 0.12) over the ac/w range 0.50–0.83.

The above considerations imply that the NH fragments of the tritylamines, imagining for a moment that a real separation were indeed feasible, in the range indicated would virtually travel along with the eluent front thereby undergoing an increasing hydration to compensate for the lowering effect of a diminishing ϱ factor on the "naked" τ(NH) value.

In this imaginary separation procedure, the remaining parts of the molecule would hardly show any tendency to become detached from the starting line of the thin-layer plate.

To illustrate this in more detail, we give below a fairly complete elaboration of the $R_M/\Sigma f$ regression equation valid for the ac/w ratio of 0.75:

$$R_M = 0.220\ \Sigma f - 0.352 \tag{43}$$

* In any case, this is how it is in theory. However, what is observed in practice is a slowly moving front attended by a fissuring followed by a crispation of the oil-impregnated silica gel layer.

TABLE 5

SUMMARY OF FRAGMENTAL VALUES IN THE RPTLC SYSTEMS INVESTIGATED

ac/w	*ϱ*	*Δh*		*f (NH)*	*τ(NH)*
		R_M scale	*Log P scale*		
0.50	0.422	−0.938	−2.22	−4.03	−1.70
0.56	0.380	−1.236	−3.25	−5.06	−1.92
0.67	0.297	−1.276	−4.30	−6.11	−1.81
0.75	0.220	−1.201	−5.46	−7.27	−1.60
0.82	0.181	−1.302	−7.19	−9.06	−1.64
0.83	0.164	−1.491	−9.09	−10.90	−1.79
0.91	0.160	−1.907	−11.92	−13.73	(−2.19)

f values:

Alkyl substituent: $f = 0.701 + (n - 1)\,0.519$

$\varnothing_3C$: $f = 5.675$

NH: $f = -1.814$

Δh factor: $f = -5.459$

τ values:

Alkyl substituent: $\tau = 0.220\,[0.701 + (n - 1)\,0.519] = 0.154 + (n - 1)\,0.114$

$\varnothing_3C$: $\tau = 0.220 \cdot 5.675 = 1.249.....$

NH ("naked"): $\tau = 0.220 \cdot -1.814 = -0.399$ } -0.351

Δh factor: $\tau = 0.220 \cdot -5.459 = -1.201$ } (= intercept)

$\tau(NH)_{hydrated} = -1.600.....$

$\Sigma\tau$ for the lipophilic parts of tritylamines ranges from 1.403 (C_1) to 2.201 (C_8).

$\langle R_F \rangle$ values (where the symbol $\langle\ \rangle$ denotes the imaginary R_F values of fragments):

$\langle R_F \rangle_{lip.\,total}$ ranges from 0.038 (C_1) to 0.006 (C_8)

$\langle R_F \rangle_{NH(hydrated)} = 0.975$

A closer look at the RPTLC behavioural pattern of other functional groups, such as OH and COO, appears necessary before any further consequence from the proposed R_M fragmentation can be envisaged. Besides, it would seem of much interest to extend the developed views to a number of high-performance liquid chromatographic experiments.

7. SUMMARY

A few years ago we introduced the hydrophobic fragmental constant, *f*, in order to promote a better understanding of lipophilicity values measured by partition measurements. A similar R_M fragmentation is now proposed for reversed-phase thin-layer chromatographic data. These fragmental values allow a closer examination of Collander-type regressions and open the way for a re-interpretation of the intercept terms of these regressions.

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