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CHROMATOGRAPHIC HYDROPHOBIC PARAMETERS IN CORRELATION ANALYSIS OF STRUCTURE–ACTIVITY RELATIONSHIPS

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CONTENTS

1. INTRODUCTION

Recent advances in the correlation of chemical structure with biological activity have demonstrated the effectiveness of using quantitative models of structure–activity relationships in describing drug action[1]. Such models, which attempt to relate the physicochemical description of a drug —by means of "critical molecular properties"[2] or parameters— to its activity, have two primary objectives. Firstly, to provide

an insight into how and why a particular drug has its effect, and secondly, to predict efficiently chemical structures of drug candidates having higher therapeutic effectiveness.

Such progress in the use of quantitative structure–activity relationships (QSAR) has shown the importance of the hydrophobic or lipophilic nature of drugs. The hydrophobicity of a drug (that is, the tendency of a species to be readily soluble in most non-polar solvents, but only sparingly soluble in water[3]), is usually characterized by the partition coefficient, P, obtained from distribution studies of the drug between an immiscible polar and "non-polar" solvent pair. The work of Martin and Synge[4] and of Consden *et al.*[5] in establishing relationships between the R_F values obtained from partition chromatography and the partition coefficient, has led to the limited use of hydrophobic parameters obtained from chromatographic measurements in QSAR models. In reviews and studies of QSAR models, many authors (see, *e.g.*, refs. 6 and 7) when briefly mentioning chromatographic parameters, normally comment that R_M values will find an increasing importance in future QSAR studies, either directly in correlation, or as a means of estimating log P or π (ref. 8) values.

The aims of this review are: (i) to discuss the measurement of such parameters and show how they are theoretically and experimentally related to other free-energy based parameters, (ii) to bring together successful correlations of R_M with biological/biochemical systems, and (iii) to suggest that the chromatographically obtained parameter should have wider applicability in structure–activity relationships.

2. FREE-ENERGY RELATIONSHIPS AND BIOLOGICAL/BIOCHEMICAL ACTIVITY

The semi-empirical approach of Hammett[9], in 1937, in correlating reaction rates for side-chain reactions in substituted aromatic compounds, by use of a linear free-energy approach[10], provided a means of quantifying the chemical structure of a molecule, and relating it to its chemical reactivity. Such an approach has enabled the medicinal chemist[1,2] to correlate the physico-chemical description of a drug with its biological or biochemical activity.

Hammett first suggested that an equation of the form

$$\log \frac{k}{k_0} = \varrho\sigma \tag{2.1}$$

might be employed to correlate the influence of *meta* and *para* substituents on the reactivity of substrates containing aromatic groupings. σ and ϱ were defined as the substituent and reaction parameter, respectively. Eqn. 2.1 is now recognised as an example of a linear free-energy relationship (LFER). These may be regarded[10] as linear relationships between the logarithms of the rate or equilibria constants for one reaction series, k_i^B, and those for a second reaction series k_i^A, subjected to the same variations in reactant structure or reaction conditions. The relationship is shown by eqn. 2.2.

$$\log k_i^B = m \log k_i^A + c \tag{2.2}$$

where m is the slope and c the intercept of the straight line obtained. The logarithm

of an equilibrium constant K is proportional to the standard free-energy change, ΔG^0, accompanying the reaction, *i.e.*

$$\log K = \frac{-\Delta G^0}{2.303\ RT} \tag{2.3}$$

where R and T have the usual meanings. According to the transition state theory, a specific rate constant, k, can be expressed in terms of a standard free energy of activation, $\Delta G^{\ddagger}$, so

$$\log k = \log \frac{RT}{Nh} - \frac{\Delta G^{\ddagger}}{2.303\ RT} \tag{2.4}$$

where N and h are the Avogadro number and Planck's constant, respectively. Combination of eqns. 2.2, 2.3 and 2.4 results in

$$\Delta G^{\mathrm{B}} = n\Delta G^{\mathrm{A}} + d \tag{2.5}$$

where the relationship between n and m, and between d and c, depends on whether the comparison between reactivity is expressed in terms of equilibrium constants or rate constants, or both. Thus, the empirical correlation of reactivity change (eqn. 2.2) is equivalent to a linear free-energy relationship, that is, to eqn. 2.5.

Of all the possible relationships between observable quantities, the rectilinear form (eqn. 2.2) is the most easily recognised. This is particularly the case when data are examined graphically, although it is now common practice[11,12] to use statistical multiple regression methods of analysis in correlation studies. The correlation of multiple variables, as shown in eqn. 2.6, is more difficult to visualise (three-dimensional plots would be required), but is readily handled by statistical methods.

$$\log k_i^{\mathrm{B}} = m \log k_i^{\mathrm{A}} + m' \log k_i^{\mathrm{A}'} + c \tag{2.6}$$

Here it is important to realise that eqn. 2.6 cannot correlate the data less well than eqn. 2.2. If the term $m' \log k_i^{\mathrm{A}'}$ is regarded simply as a correcting factor for eqn. 2.2, then eqn. 2.6 must give a better correlation unless $\log k_i^{\mathrm{A}'}$ has no relationship with the deviations obtained from eqn. 2.2. Statistical procedures are available to determine whether eqn. 2.6 is a significant improvement on eqn. 2.2, and whether or not there is a "real" relationship between $\log k_i^{\mathrm{B}}$ and both $\log k_i^{\mathrm{A}}$ and $\log k_i^{\mathrm{A}'}$. Multiparameter correlations require a more critical assessment than two-parameter correlations. Additional parameters inevitably improve the correlation without necessarily providing more information. Consequently, additional parameters must be shown to be orthogonally distinct from others[13]. In correlations given in this review, each parameter has been shown to be statistically significant, except if indicated otherwise.

By far the most widely known and used LFER approach in structure–activity correlations is that due to Hansch[1] and Hansch and Fujita[14]. The mathematical, stochastic approach by Hansch and his co-workers has been to factor the effects of substituents on the rates of equilibria constants into free energy terms, following on from eqn. 2.3. Their working hypothesis has been that, to a first approximation, the free energy change in a standard biological response, ΔG^0_{BR}, which can be attributed

to a single chemical or physical reaction, may be factored as follows

$$\Delta G^0_{BR} = \Delta G^0_{L/H} + \Delta G^0_{elect.} + \Delta G^0_{steric} \propto \ln k_{BR} \tag{2.7}$$

where $\Delta G^0_{L/H}$ represents that part of the free energy change which can be attributed to hydrophobic bonding, $\Delta G^0_{elect.}$ represents an electronic component, and ΔG^0_{steric} represents the spatial demands of reactants and products on the free-energy change. Using the extrathermodynamic approach of Leffler and Grunwald[15], it is possible to evaluate the substituent effects of k_{BR}. An extrathermodynamic approach may be described[15] as one using relationships not directly resulting from the principles of thermodynamics, in the sense that detailed mechanisms need not be explicitly identified. Eqn. 2.8 exemplifies this approach.

$$\log BR = \log \frac{1}{C_x} = k\pi + \varrho\sigma + k'S + k'' \propto \delta_x \log BR \tag{2.8}$$

where C_x is the molar concentration of a derivative x producing an equivalent biological or biochemical response, under standard conditions. π, σ and S are extrathermodynamic substituent constants for the respective effects described by eqn. 2.7.

From many studies, Hansch[1] argued that the change in the free energy of a biological response due to the hydrophobic nature of the drug might be represented by log P, π, R_M, ΔR_M, and under certain conditions parachor. The $\Delta G^0_{elect.}$ term may be factored using the various forms of the Hammett substituent parameter[10], by dipole moments, or by quantum mechanically calculated electron densities, etc. Similarly, the ΔG^0_{steric} term can be represented by such terms as molar volume, etc. However, because of the overwhelming contribution of the $\Delta G^0_{L/H}$ term over the other terms of eqn. 2.7 towards the biological response, it is on the hydrophobic parameter that most attention is focused in correlation studies.

It should be noted that such parameters can be used in QSAR models other than free-energy relation techniques analysed by regression analysis[16], and also in quantitative models unrelated to the LFER method of analysis[17]. Their so far limited uses, and the non-availability of results obtained by using chromatographically derived parameters, precludes their further discussion in this review.

3. HYDROPHOBICITY AND THE PARTITION COEFFICIENT, AND R_M PARAMETERS

By classical definition[18], a hydrophobic "bond" is formed when two or more non-polar groups in an aqueous medium come into contact, thus decreasing the extent of interaction with the surrounding water molecules, and resulting in the liberation of water originally bound by the molecules. The hydrophobic bond is recognised to be complex in nature, involving polar and apolar interactions; the hydrophobic bond concept has been useful in rationalizing biochemical phenomena[18–20], and has been applied[21] in explaining association of organic and biologic molecules in aqueous solution. In QSAR models, the ability of a compound to partition between a relatively non-polar solvent and water is normally used as a measure of its hydrophobic character.

The partition coefficient and R_M value are free-energy based terms in the thermodynamic description of the partitioning process. It is pertinent to this review to

describe the theoretical relationship between the free energy of transfer of a molecule from an aqueous to an apolar phase and its partition coefficient (or R_M), and although the treatment that follows is for completely immiscible solvent pairs, Leo *et al.*[22] have shown that where there is some mutual solubility in the cases of the solvent pair, the derived relationship still holds.

An ideal solution may be defined[23] as one in which each component follows the equation

$$\mu_i(T,Pr,X) = \mu_i^0(T,Pr) + RT \ln X_i \tag{3.1}$$

where μ_i^0 is the chemical potential of pure component i in solution at specified temperature (T) and pressure (Pr), and X_i is its mole fraction. (If the solution was to become non-ideal before $X_i = 1$, then μ_i^0 ceases to be the actual chemical potential of pure i, and has the value it would have if the solution remained ideal up to $X_i = 1$).

Cratin[24] has shown that, as a consequence, the thermodynamic partition coefficient P', based upon ideal solution behaviour, should have the form

$$P' = \frac{X(\mathrm{w})}{X(\mathrm{o})} \tag{3.2}$$

in which X(w) and X(o) refer to the mole fraction of drug in the aqueous and non-aqueous phases, respectively. Cratin has further demonstrated that for dilute solutions eqn. 3.1 may be rewritten for component i in the following way:

$$\mu_i(T,Pr,X) = \mu_i^0(T,Pr) + RT \ln \bar{V}_s^0 + RT \ln C_i \tag{3.3}$$

Eqn. 3.3 shows that the chemical potential based upon mole fractions, is larger than that based upon the molar concentrations, by $RT \ln \bar{V}_s^0$, where $\bar{V}_s^0$ is the molar volume of solvent in the solution. Such a relationship means that the value of the chemical potential if expressed on the molar concentration scale, even for ideal solutions, depends upon the molar volume of the solvent.

During the partitioning process between an immiscible solvent pair, it can be assumed that the free energy of transfer of a molecular species can be factored due to the contributions of its constituent parts. Assuming that the total free energy of a molecule, μ_T, is comprised of a lipophilic group (L) and "n" hydrophilic groups (H), then the total transfer free energy may be represented by the equations

$$\mu_T(\mathrm{w}) = \mu_L(\mathrm{w}) + n\,\mu_H(\mathrm{w}) \tag{3.4}$$

and

$$\mu_T(\mathrm{o}) = \mu_L(\mathrm{o}) + n\,\mu_H(\mathrm{o}) \tag{3.5}$$

where (w) and (o) again refer to the aqueous and non-aqueous phases, respectively. Assuming ideal behaviour (*i.e.*, eqn. 3.1), and converting from mole fraction terms to concentration terms, then the above equations become

$$\mu_T(\mathrm{w}) = \mu_L^0(\mathrm{w}) + n\,\mu^0(\mathrm{w}) + RT \ln \bar{V}^0(\mathrm{w}) + RT \ln C(\mathrm{w}) \tag{3.6}$$

and

$$\mu_T(o) = \mu_L^0(o) + n\mu_H^0(o) + RT \ln \bar{V}^0(o) + RT \ln C(o) \quad (3.7)$$

At equilibrium $\mu_T(w) = \mu_T(o)$ and eqns. 3.6 and 3.7 may be equated as follows

$$[\mu_L^0(w) - \mu_L^0(o)] + RT \ln \frac{\bar{V}^0(w)}{\bar{V}^0(o)} + n[\mu_H^0(w) - \mu_H^0(o)] = -RT \ln \frac{C(w)}{C(o)} \quad (3.8)$$

By replacing $C(w)/C(o)$ by P and putting

$$\Delta\mu^0 = \mu^0(o) - \mu^0(w)$$

the following expression is obtained

$$\log P = \frac{n\Delta\mu_H^0}{2.303\,RT} + \frac{\Delta\mu_L^0}{2.303\,RT} + \log \frac{\bar{V}^0(o)}{\bar{V}^0(w)} \quad (3.9)$$

For eqn. 3.9 to be valid a plot of $\log P$ *vs.* n should be a straight line with a slope equal to $\Delta\mu_H^0/2.303RT$. Using the relationship between the logarithm of the partition coefficient *versus* the number of ethylene oxide adducts in *p-tert.*-octylphenols, found by Crook *et al.*[25], where the regression equation for the relationship is

$$\log P = 0.422\,n - 3.836 \quad (3.10)$$

a standard free-energy change of transfer per mole of ethylene oxide [(o) → (w)] of −2.51 kJ (at 25°) can be found. A similar treatment of solute behaviour should be applicable to information collected from chromatographic measurements. The data collected by Green *et al.*[26] (Fig. 1) of R_M values for *n*-alkyl dinitrobenzoates,

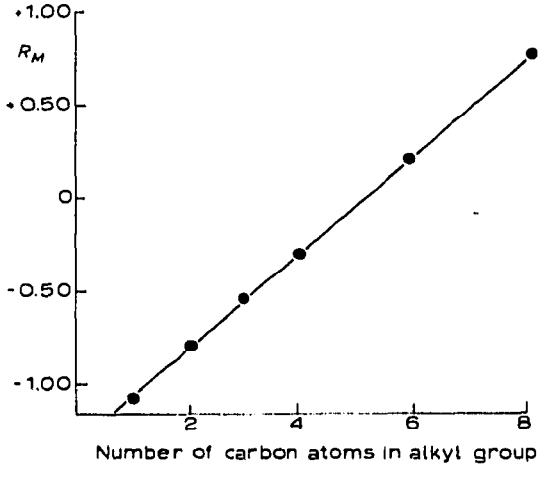


Fig. 1. Relationship between the R_M values and the number of carbon atoms of a series of *n*-alkyl dinitrobenzoates. (After Green *et al.*[26].)

measured in a paper reversed-phase system of liquid paraffin–50% aqueous ethanol, illustrate how when log P is substituted by R_M in eqn. 3.9, the derived theoretical relationship is still valid. A regression slope of +0.245 is obtained which gives a standard free energy of transfer per methylene group of 1.39 kJ (at 25°), though for comparison purposes with other $\Delta(\Delta G_{(CH_2)})$ values[27] it should be remembered that this is for the transfer of a methylene group from a non-polar to an aqueous ethanol environment.

It should be seen that other group free energies can be determined using an approach similar to that used by eqn. 3.9 (and illustrated by eqn. 3.10 and Fig. 1).

The theoretical basis for the relationship between R_F values in partition chromatography and chemical structure was first proposed by Consden *et al.*[5], and later by Martin[28], who deduced that for ideal solutions the partition coefficient, P, of a substance A between two phases is related to the free energy required to transport one mole of A from one phase to another by the expression.

$$\ln P = \frac{\Delta\mu_A}{RT} \qquad (3.11)$$

Martin showed that the addition of a group X to the substance A should change the partition coefficient by a factor depending only on the nature of X and the two phases, although not on A itself. Hence, if A is substituted by "n" groups X, "m" groups Y, etc., then

$$RT \ln P = \Delta\mu_A + n\Delta\mu_X + m\Delta\mu_Y + \ldots \text{ etc.} \qquad (3.12)$$

(In a similar way to eqns. 3.4 and 3.5, X and Y could represent hydrophilic and lipophilic moieties). Since

$$P = \frac{A_m}{A_s}\left(\frac{1}{R_F} - 1\right) \qquad (3.13)$$

where A_m/A_s is the effective ratio of the cross-sectional areas of the mobile and stationary phases, then

$$RT \ln \frac{A_m}{A_s}\left(\frac{1}{R_F} - 1\right) = \Delta\mu_A + n\Delta\mu_X + m\Delta\mu_Y + \ldots \text{ etc.} \qquad (3.14)$$

Bate-Smith and Westall[29] introduced the term

$$R_M = \log\left(\frac{1}{R_F} - 1\right) \qquad (3.15)$$

and showed experimentally that the relationship predicted by Martin was followed for a number of flavones, anthocyanins and some related compounds. However, because of the nature of the substituent groups studied (for example, hydroxyl groups), data were necessarily restricted to a limited range of compounds.

The partition coefficient can be defined as an equilibrium constant, such that $k_a/k_b = P$. In doing so it is reasonable to express the effect of a given function on the

partition coefficient of a parent molecule in terms of

$$\log \frac{P_X}{P_H} = \pi k_p \tag{3.16}$$

where k_p will be a constant depending on the nature of the phases employed in the measurement of $\log P$, $\log (P_X/P_H)$ is proportional to the difference in free-energy changes involved in transferring unsubstituted and substituted molecules from one phase to another, and P_X and P_H represent the partition coefficients of the substituted and unsubstituted molecules, respectively.

Assuming that for any given standard system k_p is unity, then

$$\pi_X = \log P_X - \log P_H \tag{3.17}$$

where, when $\log P_H + \pi$ is zero, the free-energy change in moving from one phase to the other is zero. π is conceptually regarded as constant for a given functional group and represents that part of the transfer free-energy change due to any particular group or function, that is

$$\Delta(\Delta G^0) = \Delta G_X^0 - \Delta G_H^0 = -RT \ln P_X + RT \ln P_H \tag{3.18}$$

Therefore

$$\ln P_X - \ln P_H = \frac{-\Delta(\Delta G^0)}{RT} \tag{3.19}$$

and

$$\log \frac{P_X}{P_H} = k[-\Delta(\Delta G^0)] = k_p \pi \tag{3.20}$$

By substituting eqn. 3.17 into the relationship exemplified by eqn. 3.13, the following expression is obtained

$$\pi_X = \left[\log \frac{A_m}{A_s} + \log \left(\frac{1}{R_{FX}} - 1\right)\right] - \left[\log \frac{A_m}{A_s} + \log \left(\frac{1}{R_{FH}} - 1\right)\right] \tag{3.21}$$

which, in terms of R_M (eqn. 3.15), becomes

$$\pi_X = R_{MX} - R_{MH} = \Delta R_{MX} \tag{3.22}$$

that is π is analogous to ΔR_M. It is interesting to note in this respect the work of Clifford *et al.*[30] in correlating fungicidal activity with chemical constitution of some alkyldinitrophenols, in that they ignore the term ΔR_M and express π directly as

$$\pi = \log \frac{(1/R_{FX}) - 1}{(1/R_{FH}) - 1} \tag{3.23}$$

For acids or bases, R_M can be related to R_F by the following expression, providing the degree of association in the organic phase can be ignored

$$R_M = \log\left(\frac{1}{R_F} - 1\right) + \log\frac{K_A + [H^+]}{[H^+]} \qquad (3.24)$$

where K_A is the dissociation constant of the solute, and $[H^+]$ is the hydrogen ion concentration of the mobile phase.

4. MEASUREMENT OF R_M

Bush[31], in his excellent review of the R_M treatment in chromatographic analysis, has devoted a major section of the study to the classification and design of solvent systems in which one can measure R_M values. It is to this study, and to the pertinent chromatographic literature for individual solute R_M determinations, that the medicinal chemist is directed for his search of appropriate systems.

However, some of the more important experimental design variables and the relevance of obtained data deserve comment. Successful correlations of the LFER type have been made with values determined on paper and thin layers only, and accordingly this present study concerns itself primarily with such methods: other methods, such as liquid–liquid partition chromatography[32], (where the retention volume can be related to the partition coefficient), because of their more quantitative approach, and because they lend themselves to a more precise control of experimental variables, should be seriously considered in the future for providing accurate, reproducible hydrophobic parameters.

Literature R_M values can be seen to have been determined either in non-reversed (or straight) or in reversed-phase systems, and also by paper and thin-layer methods. The theory of Martin and Synge[4], and Consden *et al.*[5], and others, was derived for systems where the partition process only was taking place: however, as pointed out by Oscik[33], many workers quite automatically apply the relevant relationships derived for the partition chromatography theory to the theory of adsorption chromatography. Oscik has further stressed the fundamental differences between ΔR_M values determined by either method, and has derived a thermodynamically defined term, $(\Delta\mu_s^0)_{\alpha,\beta}$, which characterizes the adsorption forces acting on the molecules of the solute and the two organic solvents used, and which may be employed to describe the basic differences between partition and adsorption chromatography. It is therefore important to make R_M determinations in systems where partition either is the sole process taking place or predominates others. The recent study by Plá-Delfina *et al.*[34] has recognized such considerations. In their study, relating R_M values to adsorption rate constants of some barbiturate drugs (see also section 6), they were faced with literature R_M data reported for thin-layer systems, using activated plates without impregnation where adsorption mechanisms are at least as important as partition processes, and paper chromatographic data where partition mechanisms are thought to prevail. By chosing the data from the latter systems, successful correlations were obtained.

In non-reversed conditions, paper methods are comprised of the following physico-chemical mechanisms, *viz.* adsorption, desorption and solvation (followed by elution). Janardhan and Paul[35] have demonstrated that the mobile phase in paper chromatography requires proton availability for adsorption, and desorption processes to occur, and that in the absence of $[H^+]$ a kind of diffusion occurs as a result of

partition and a differential distribution of the mobile phase —thus affecting R_M values. Ion-exchange processes are also known to occur in non-reversed phase systems[36].

With thin-layer methods, it is important to achieve complete saturation of the tank system because of possible temperature dependence of the R_M value, due to changes in activity (which becomes greater at lower temperatures). It is recommended that the general procedures given by Dallas[37] are followed when using the plate method. Problems can arise with polar ionised drug molecules, or in systems where polar reversed phases are used. Bark *et al.*[38] have shown that causes of variation in solute distribution can be due to interaction between materials of the two phases as the molarity of the acid used as the developing solvent is increased, and that R_F values vary significantly with the flow-rate (but not flow geometry) of the developing solvent. Green and Marcinkiewicz[39], commenting upon their extensive paper chromatographic studies relating R_F and R_M values to chemical constitution, advise upon the use of horizontal, tankless methods using reversed-phase systems —mainly because equilibration difficulties are avoided and there is good replication of result. Green and McHale[40] add that if paper chromatograms are developed in tanks, then the length of the descending run must be carefully controlled; and, here also, reversed-phase systems are advantageous since the character and constitution of the stationary phase is more clearly defined and equilibration is of less importance. (Though see the findings of Dallas, above). Green and McHale also point to the findings of Bush[41], who stated that the chromatographic system must give R_F values between 0.2 and 0.8 in order for confidence to be placed upon the determined value.

In the measurement of R_M values by reversed-phase methods, systems developed often consist of paper or thin-layer absorbent impregnated with a lipophilic phase (*e.g.* light paraffin, ethyl oleate, 1-octanol) and an aqueous mobile phase of varying constitution and polarity.

Because of the nature of some of the solutes examined, it is often found neces-

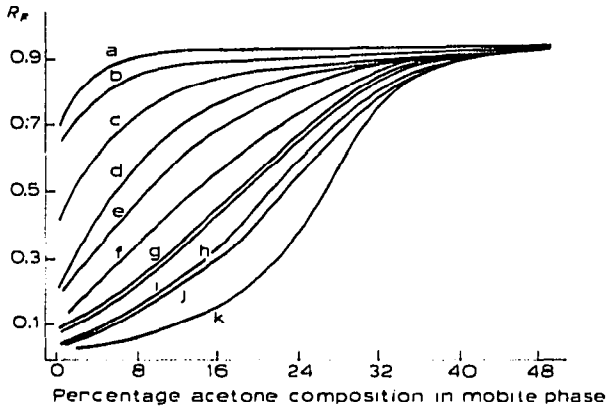
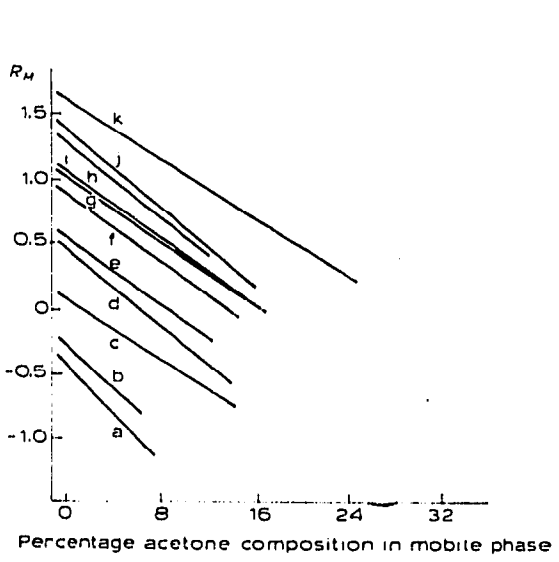


Fig. 2. Experimental curves for the relationship between R_F values and percentage acetone composition in the mobile phase for some penicillins studied using reversed-phase chromatography. (After Biagi *et al.*[42].) a = Carboxypenicillin; b = methylenampicillin; c = ampicillin; d = methicillin; e = benzylpenicillin; f = phenoxymethylpenicillin; g = phenethicillin; h = oxacillin; i = chloxacillin; j = nafcillin; k = dichloxacillin.



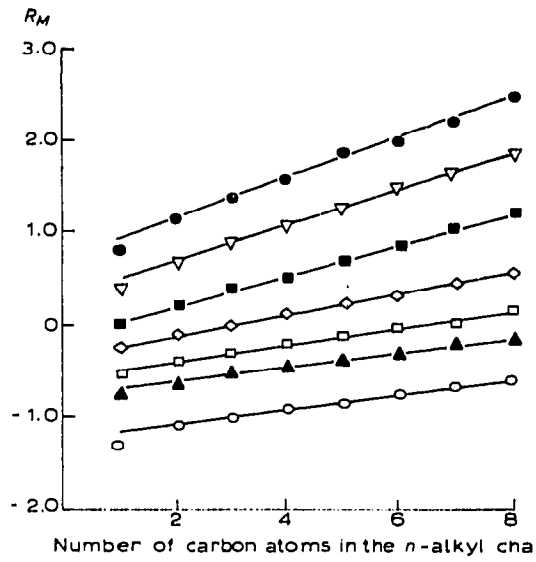


Fig. 3. Linear portions of the relationships between R_M values and percentage acetone composition derived from the data shown in Fig. 2. (After Biagi *et al.*[42].)

Fig. 4. Relationship between the R_M values and the number of methylene carbon atoms in the alkyl chain of N-*n*-alkyltritylamines in a series of acetone–water mixtures. The proportion of acetone in the mobile phases is: ●, 0.50; ▽, 0.56; ■, 0.67; ◇, 0.75; □, 0.80; ▲, 0.83; and ○, 0.91. (After Boyce and Milborrow[7].)

sary to increase or decrease the polarity of the mobile phase in order to achieve reasonable migration of the solute and so obtain measurable R_F values. For example, the addition to the aqueous mobile phase of acetone or acetone–dioxan mixtures is frequently made. Such a technique, which results in a consequent change in the R_M value, is now fairly common in the literature, for example, the studies of Biagi *et al.*[42] in correlating the effect of acetone concentration in the mobile phase on R_F and R_M values (Figs. 2 and 3) for a series of penicillin drugs.

Boyce and Milborrow[7] have also shown linear relationships between R_M values and the number of methylene carbon atoms in the *n*-alkyl chain of N-*n*-alkyltritylamines, in a series of acetone–water systems (Fig. 4). Allied to this are the early findings of Isherwood[43], who related the water content of the mobile phase to the R_M values of oligosaccharides, when measured by partition methods.

Such evidence of a mobile phase composition effect on R_M data questions the significance of reported R_M and ΔR_M values measured in similar systems when being compared to one another. Correctly, Biagi and his co-workers in recent reported work cite R_M data for values found by extrapolation of the acetone percentage compositio.. *vs.* R_M curves (in for example Fig. 3) to the *y*-axis, and these theoretical R_M values derived for a 100% water/non-polar system are then used for any intended QSAR model. Such extrapolation of data has been shown to be theoretically and experimentally correct by Soczewiński and Wachtmeister[44], who demonstrated that R_M values for a compound in some ternary two-phase systems can be shown to be linear

functions of the volume composition of the binary mobile phase. R_M and mobile phase composition are related by the following expression

$$R_M = \varphi_1 R_{M1} + \varphi_2 R_{M2} \tag{4.1}$$

where φ_1 and φ_2 are the volume fractions of the components in the binary solvent phase and R_{M1} and R_{M2} are the R_M values for the solute found using pure component 1 and pure component 2. (Non-linearity of the expression can sometimes occur with extremely polar phases due to volume effects upon mixing[31].)

A similar effect has been reported by Soczewiński and Kuczyński[45], who presented findings on the developing solvent composition compared to R_M and log C_s (log molar solubility of the solute) for various alkaloidal solutes on buffer-impregnated paper. More importantly, they found that the differences in R_M and log C_s values were individual for a given solute/solvent system, even for those solutes where ΔR_M and Δlog C_s values tend to be constant for various systems. Recent work by Oscik and Rozylo[46] has demonstrated the use of a derived equation relating the values of R_M coefficients measured by adsorption techniques with the composition of a two-component mobile phase. This equation enables theoretical R_M values to be generated for the situation in which pure solvents are used as the mobile phases.

Similar attention should be given to the nature of the other member of the solvent pair. In reversed-phase systems impregnation of the paper or thin-layer absorbent support is usually done using organic solvents of varying polarity and it is wrong to discuss them on terms of "non-polar" phases.

Partition coefficients may be regarded as equilibrium constants and as such there should be extrathermodynamic relationships[15] between partition coefficients measured in different solvent systems. Although R_M values are not obtained from true equilibrium parameters, they can be regarded as being derived from steady-state functions, and as such may be expected to show these same extrathermodynamic relationships. Collander[47], in finding that ether–water and olive oil–water partition coefficients were equally well correlated with penetration into *Nitella* cells, pointed out that the nature of the organic phase should not affect the results qualitatively, and expressed his findings in the following manner

$$\log P_2 = a \log P_1 + b \tag{4.2}$$

i.e., rectilinear relationships exist between partition coefficients found in one system (P_1) and those found in a second (P_2), providing the polar phase is water, and the non-aqueous phases contain the same functional group. Collander was further able to show that eqn. 4.2 was of significant value when comparing the systems isobutanol–water, isopentanol–water, octanol–water, and oleyl alcohol–water. Leo and Hansch[48] and Leo *et al.*[22] have comprehensively extended the Collander expression to many other partitioning solvent systems and have shown that eqn. 4.2 holds well when P_1 and P_2 are found using similar non-aqueous solvents, such as alkanols, esters and ethers, but that it breaks down when comparisons are attempted between hydrocarbons (such as cyclohexane) and solvents with hydrogen-bonding ability such as alkanols, esters, etc. It is necessary in such cases, when attempting to derive theoretical relationships between, for example, heptane and 1-octanol, to generate two regression equa-

tions, one relating to "acidic" solutes and the other to "basic" solutes, depending on their hydrogen ion acceptor or donator abilities. Similar arguments should hold for R_M determinations.

A small number of workers have reported the derived relationships existing between R_M values measured in various systems. Lien *et al.*[49], using Bakerflex sheets pre-coated with silica gel 1B and two solvent systems, *viz.* dioxan and butanol–acetic acid–water (4:2:1), were able to give derived regression equations for the relationships between R_M values of some thiolactams measured in the two systems. The equation is shown as eqn. 4.3 in Table 1.

Table 1 gives reported equations from the scientific literature and elsewhere, showing the statistical relationships existing between R_M values of drug molecules measured in various systems. Eqn. 4.4 is the equation derived by Biagi *et al.*[50] for some testosterone esters, where the R_M value has been found using a reversed-phase thin-layer technique, with the stationary silica gel G layer being impregnated by a 5% silicone oil solution (in ether). The polar mobile phase was either an acetone–water or a methanol–water system of varying composition. Although the percentage composition of the acetone ranged from 42 to 74% and that of methanol from 54 to 86%, the shown equation is derived for the R_M values generated at a 54% organic component composition. Similar order of correlation is reported by Draber *et al.*[51] in comparing R_M values of some substituted triazinone herbicides, measured in a system comprised of paraffin oil on silica gel (NHR type) thin-layer plates and water–dioxan–acetone (13:10:7); and in a system of commercial polyamide plates with water–dioxan–acetone (2:1:1) as the mobile phase. The reasonable correlation obtained by these workers (eqn. 4.5) surprisingly indicates that Collander's relationship (eqn. 4.1) can apply to situations where both the mobile and stationary phases are changed simultaneously (though in the case of the mobile phase only by percentage composition).

Dearden and Tomlinson[52], in a study relating ΔR_M values to the biological activity of some *p*-substituted acetanilides (see also Section 6), report the ΔR_M values for the *para* substituents found in, again, a silica gel thin-layer reversed system impregnated using one of either two non-aqueous solvents, liquid paraffin or 1-octanol. The mobile phase used was acetone–water (20% v/v acetone for liquid paraffin, 10% v/v acetone for 1-octanol). This relationship is also shown in Fig. 5.

Tomlinson[53] has further demonstrated (eqn. 4.7) the usefulness of Collander's expression, by including into the regression analysis embodied by eqn. 4.6 two acetanilides substituted in the *ortho* position and in which intramolecular bonding is expected with one of them. Although the correlation coefficient falls, a variance-ratio test analysis reveals both equations to be significant at the same high level, indicating that at least in this case ΔR_M *ortho* values are constant from one system to another.

The paucity of data in the literature of the type shown in Table 1 should be noted and rectified, and, although other data have been presented in graphical form, it is hoped that as more experimental data are generated, statistically derived equations of the type shown in Table 1 will be given in the literature.

In this way standard regression equations can be obtained, so that computation of preferred R_M data in any chosen standard system can be made in a similar way as has been carried out for partition coefficients[22,48].

TABLE I

DERIVED REGRESSION EQUATIONS FOR RELATIONSHIPS BETWEEN R_M AND ΔR_M VALUES, FOUND FOR VARIOUS SYSTEMS

All equations have reversed-phase notation. For measurements used to derive eqns. 4.3 and 4.4, the stationary phase was kept constant. For eqns. 4.5–4.7, the stationary phase was altered. *n*, *r*, and *s* are the number of data points, the correlation coefficient, and the residual sum of squares for the shown relationships, respectively.

Solute	*Regression equation*	*n*	*r*	*s*	*Eqn.*	*Ref.*
Thiolactams	$R_{M(BAW)} = 1.604\ R_{M(dion)} - 0.606$	5	0.966	0.041	4.3	49
Testosterone esters	$R_{M(Me_2CO)} = 0.728\ R_{M(MeOH)} - 0.509$	14	0.993	0.051	4.4	50
Triazinones	$R_{M(polyamide)} = 2.049\ R_{M(NHR)} + 0.401$	32	0.964	0.109	4.5	51
para-substituted acetanilides	$\Delta R_{M(paraffin)} = 0.687\ \Delta R_{M(oct)} - 0.031$	16	0.970	0.104	4.6	52
para- and *ortho*-substituted acetanilides	$\Delta R_{M(paraffin)} = 0.651\ \Delta R_{M(oct)} - 0.006$	18	0.956	0.173	4.7	53

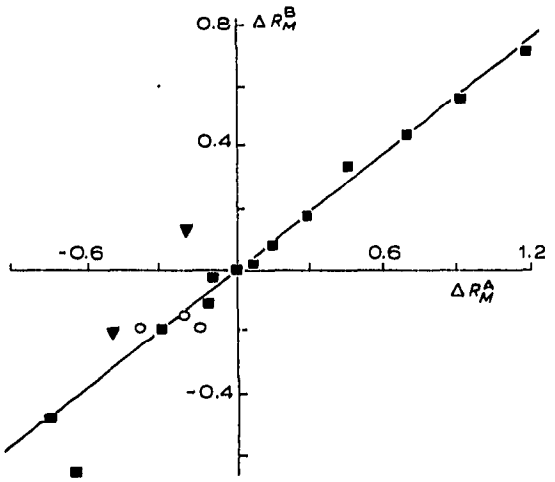


Fig. 5. Relationship between two sets of ΔR_M constants for a series of substituted acetanilides, as measured in a 1-octanol/acetone–water (1:9) system (A), and in a liquid paraffin/acetone–water (2:8) system (B). ■, *para* substituted compounds; ▼, *ortho* substituted compounds; ○, N-methyl-substituted acetanilides (Tomlinson[53]).

Apart from already discussed considerations, there follows a brief summary of the advantages of chromatographic methods over direct partition methods for obtaining an index of hydrophobicity, as discussed by various authors:

(1) Simple to use, rapid and less tedious. For example[7], up to 25 different solutes can be developed simultaneously on a thin-layer plate, so enabling a direct comparison of R_M values to be made.

(2) Little material needs to be used. This may be extremely important in the future when considering hydrophobicity of molecules of biological origin.

(3) Chromatographic methods are able to accommodate drug molecules of very high or low P value. Such solutes require a long equilibration in normal "shake-flask" methods, and the solvent pair ratios required may preclude their measurement with such automated techniques as continuous solvent extraction[54].

(4) The material to be examined need not be ultrapure, for impurities are normally separated during development.

(5) There is no need for a quantitative analysis of the solute.

(6) More reproducible results are usually found over those derived from direct partition coefficient techniques (*e.g.*, refs. 7 and 53).

(7) Reversed-phase paper or thin-layer chromatography in a range of solvent mixtures can give R_M values for any of these mixtures provided that the linear relationship between solvent composition is established, so enabling R_M values in a chosen standard system to be derived.

Two relevant disadvantages of the methods are that "streaking" of spots is sometimes unavoidable, especially in reversed-phase systems, due to overloading of solute to obtain visualization, and that this effect, coupled with poor visualization, increases any subjective errors made when measuring the R_F values. Also, in reversed-

phase systems again, an even distribution of the non-aqueous phase upon impregnation of the support is not known for certain. This could affect the R_M value, though replication should overcome this.

5.1 R_M CONSTANTS AND THE RELATIONSHIPS BETWEEN CHROMATOGRAPHICALLY DERIVED PARAMETERS AND OTHER FREE-ENERGY BASED PARAMETERS

Additivity of hydrophobic substituent constants will be possible when the relationship embodied in eqn. 3.9 is obeyed. If log P is substituted by R_M, then a regular and constant increase in R_M over a parent structure should occur if the parent molecule is polysubstituted by a constant group. This is demonstrated by Fig. 1, in which the methylene group is substituted into a *n*-alkyl dinitrobenzoate structure. This effect is basic to the theoretical treatment of R_M data by Martin[28], that is, the R_M parameter is constituted of the R_M values of its component parts, and that these values are additive.

$$R_{MB} = R_{MA} + R_{MX} + R_{MY} + R_{MZ} \tag{5.1}$$

It may be seen from eqn. 3.14 that such a relationship can also be expressed in free-energy terms. Bush[31] has shown that if all the component parts were equivalent and thus had equal R_M values, then the R_M of the molecule B may be written as

$$R_{MB} = \sum_{x=A}^{x=Z} \Delta \log P_x - \log \frac{A_m}{A_s} \tag{5.2}$$

where x is the equivalent component. The $\log A_m/A_s$ term is used when experimental or theoretical determination of R_M values is needed for a series of compounds, for which no reference R_M values for the series are known. However, it is usual practice in R_M value prediction for one reference R_M value to be known and eqn. 3.22 to be used for calculating the required value, knowing the ΔR_M values of the substituent compounds.

There is abundant evidence in the literature that the additivity rule does not always hold, and consequently estimated R_M values do not equal experimental values, that is, $R_M \neq \Sigma \Delta R_M$. Similar non-additivity can be demonstrated for log P using certain π values[55]. This is not surprising when one considers that ΔR_M values or π values, used to predict the respective R_M or log P parameter, are those usually obtained from non-interacting systems where there is more likelihood of the constancy of the substituent constant. Table 2 gives ΔR_M values obtained from the literature for various important groups. Where possible values are given for the group when it is in an interacting and also when it is in a non-interacting environment. Fujita *et al.*[8] found that the π value for an alkyl group was virtually independent of the system in which it was measured but, for more polar and especially for groups able to hydrogen bond, the π value varied according to the environment in which it was determined, that is, when its character was able to be influenced by the presence of closely situated groups. Similar effects have been demonstrated for ΔR_M values by Marcinkiewicz and Green[56] and others[26,39,40]. There are now seen to be a number of causes which can lead to non-additivity of ΔR_M (or π) values and these will now be discussed.

Martin's treatment assumes that for any stated solvent system the ΔR_M change caused by the introduction for group X into a parent structure is of constant value, providing that its substitution into the parent structure does not result in any intramolecular interactions with other functions in the structure. Conversely, it can be appreciated that if the introduction of a group into a structure causes a breakdown in the additivity principle, then intra- or intermolecular effects are probably now taking place within the substituted structure.

A. Steric effects

Steric effects often account for breakdown in R_M additivity, especially so in large drug molecules containing non-planar ring systems (*e.g.* steroids[41]). However, it may be possible to overcome this, and other effects, by selecting from the literature ΔR_M values appropriate to the system under consideration. For example, Green and McHale[40] have illustrated the use of a ΔR_M increment for a single *trans*-isoprenoid unit (0.142), to predict accurately R_M values of, for example, an all-*trans*-C_{100}-isoprenoid alcohol. This approach should be used with caution if small drug molecules are considered due to the fact that the steric effect will overlap with other intramolecular interactions. Table 2 also lists some other "steric" ΔR_M increments which may be considered when R_M values need to be predicted. However, in small conjugated cyclic aromatic systems, because of the co-planarity of aromatic rings and the fact that substituents are always equatorial to the ring, such alicyclic steric effects are not evident and can be ignored. Steric effects of a type do, however, exist in non-alicyclic systems, the most common of these being the *ortho* effect.

(a) The ortho effect

When polar groups are introduced into a molecular structure adjacent or *ortho* to an established grouping, it is possible that intramolecular bonding between the two groups will occur. Such an effect has been termed the *ortho* effect. The effect can be shown[40,57] to be mainly a polar one resulting from inductive and/or mesomeric effects. In attempting to elucidate the electrical composition of π constants, Cammarata[58] has suggested that there can exist two conditions under which non-additivity of π (or ΔR_M) constants will be evident. These are: (a) when mutual electrical interaction occurs between functional groups, and (b) when a given group can no longer be desolvated to its maximum potential because of the physical effect of an adjacent or *ortho* group. Empirically, the type (a) effect can be overcome by using ΔR_M values which would be expected to have similar electrical effects. This is achieved by chosing from the literature values obtained from related solute systems and values when the studied substituent is in a similar environment. Type (b) effects will occur because of competition between two adjacent groups for the same solvated water, which is thought to exist around the molecule when in solution. Upon transfer of the substituted molecule from an aqueous to a non-aqueous phase, the desolvation process is changed, hence the entropy contribution to the transfer is altered and will result in a change in the value of the free energy term. This has the net effect, for example in a pair of isomers, in one of which the *ortho* effect is present, of reducing the R_M value in the molecule having *ortho* interactions compared to its isomer.

An example of type (a) *ortho* effect in chromatography can be demonstrated

with the data of Marcinkiewicz *et al.*[59] obtained from a study of ΔR_M effects in phenols and alkoxyphenols. From this, a calculation of the R_M value for 2,2,5,7,8-pentamethyl-6-chromanol from the R_M value for phenol and ΔR_M values for the appropriate atomic functions (see Table 2) gives a figure of +0.372, whereas experimentally a value of +0.676 is obtained. However, these workers were able to calculate from their experimental data that when *ortho* effects are thought to be present, an additional ΔR_M increment of +0.126 needs to be introduced for each affected substituent. Introduction of this value into the calculation of R_M for the substituted chromanol, in which two *ortho* effects should be occurring, produces a theoretical value of +0.624, which can be seen to be in good agreement with the experimental value. Even the ΔR_M-(*ortho*) value is not constant and can vary from system to system. For example, in a hydroquinone monoether series[59] a comparison of 4-methoxy-2-methylphenol and 4-methoxy-5-methylphenol gives an *ortho* methyl value of +0.062 (which may in this particular case be due to an electronic interaction between the 4-methoxy group and the phenolic hydroxyl group, resulting in a change in the steric effect of the methyl group). Further discrepancies can be found in the $\Delta R_{M(ortho)}$ value by examining the data in Table 2. For example, the trifluoromethyl data of Büchel and Draber[60] yield a value of +0.072. These variations, probably due to the fact that such a treatment assumes electrical interactions, are the same for *para* and *ortho*-substituents, which is not the case[61].

Type (b) *ortho* effects are commonly seen when bulky alkyl or alkoxy groups are introduced into a ring system. An example of this can be seen with the ΔR_M values for ring-attached methylene groups with simple phenol systems[59], when the $\Delta R_{M(CH_2)}$ value in a reversed-phase system goes from 0.305 to 0.220, when going from a non-interacting solute to a phenol showing methylene group "ortho effects". Such data give a $\Delta R_{M(ortho)}$ increment of +0.185. The "*ortho* effect" can also be reinforced by intramolecular hydrogen bonding.

B. Intramolecular hydrogen bonding

When such an effect occurs, the size of the deviation between $\Sigma\Delta R_M$ and R_M values is influenced by the strength of the intramolecular hydrogen bond and its free energy of formation. For example, fluorine, chlorine, or cyano groups will have no *ortho* effect due to hydrogen bonding, whereas hydroxyl and amino groups will. In aliphatic molecules or side chains, where there is α and β alkyl group substitution, intramolecularly bonded five- or six-membered rings can exist[62], so giving rise to ΔR_M changes. Effects such as this are difficult to quantify and require further study. If R_M values are strongly dependent upon the nature of the chromatographic system in which they are measured, this normally indicates that strong intramolecular bonding is taking place[59].

C. Electronic effects

Substitution into any particular system can be expected to alter the general electronic distribution of the molecule. If such disruption is great, then non-additivity may result. This effect is well documented in R_M literature[26,56,59], and may be because of the actual electronic distribution of the substituent or the effect this has on the char-

acter of the original electronic displacements in the parent molecule[40]. In aromatic systems where permanent charge separations are possible, such effects may often dominate any steric effects and will result in even larger deviations from the $\Sigma\Delta R_M = R_M$ relationship.

D. Intramolecular hydrophobic bonding

Examination of the partition coefficient data of aromatic molecules with aliphatic side chains by various workers[63,64] has revealed that for polar aliphatic substituents the π values for the polar grouping depend upon the distance of the group from the aromatic ring. Also, π values for the polar substituents, determined from completely aliphatic structures, have higher positive values than π values determined for the aromatic structures with aliphatic side-chains, where the polar group is separated from the ring by three methylene groupings. Such an effect indicates that a polar grouping such as hydroxy-, fluoro-, chloro-, methoxy-, cyano-, etc., has a higher hydrophobic nature when in a completely aliphatic system than when it is placed terminal to an aliphatic side-chain in an aromatic system.

Hansch and Anderson[64] have proposed that this effect is due to a folding of the side-chain over the phenyl ring, (the effect being assisted by the tendency of the strong dipole of the polar group to interact with the π electrons of the ring), in such a way that the polar substituent group projects away from the interaction: this would result in a more compact structure of greater water solubility, and hence a lower log P value than expected. Recent studies[65] have questioned the validity of this postulate in pointing out that on a geometric basis any interaction below an aliphatic chain of four-carbon length would result in an unfavourable strain on the structure. Whatever the answer is, the experimental facts still remain, indicating that in such situations non-additivity of ΔR_M values will occur and that direct measurement of R_M is preferable.

E. Chain-branching

Green *et al.*[26] found that compounds with branched side-chains developed faster when reversed-phase systems were used. This effect caused non-additivity of ΔR_M with substituted phenols and led to the introduction by these workers of an empirical relationship which would assist them in predicting R_M values. That is, for n branchings in a substituent chain attached to an aromatic ring system, allowance should be made for $(n-1)$ effects. There is no theoretical justification for this rule, although it can be used with some confidence. For example, the prediction of R_M values for vitamin K, ubiquinones and ubichromenols has been successfully made[26] using the $(n-1)$ rule. The ΔR_M branching effect is not affected by the length of the alkyl chain nor by the position of the branches in the chain. Bush[31] has attributed the effect to a decrease in partial molar volume over the unbranched side-chain, and also to a restriction of free rotation caused by the branching, so leading to an increased entropic effect upon partitioning. (Note, in this context, that a quaternary carbon atom is considered for purposes of the $(n-1)$ rule to consist of one branch only).

Table 2 is a compilation of ΔR_M values for various functional groups, atoms or structural effects which can be applied to the prediction of R_M values for use in QSAR models. Values have been taken from many reference sources and are quoted with a

TABLE 2

LITERATURE ΔR_M VALUES FOR VARIOUS FUNCTIONAL GROUPS, DERIVED FROM R_M VALUES MEASURED IN A WIDE RANGE OF CHROMATOGRAPHIC SYSTEMS

Functional group	*Chromatographic system****	*Solute and comments concerning the position of the functional group*	*ΔR_M value****	*Ref.*
Methylene group	Liquid paraffin/water–acetone (7:3), reversed phase, thin layer (A)	N-*n*-alkyltritylamines	+0.135 (8)	7
	Liquid paraffin/95% aq. ethanol, reversed phase, thin layer (B)	tocyl ethers	+0.113 (2)	26
	Ethyl oleate/aqueous ethanol, reversed phase, cellulose layers (C)	alkyldinitrophenols —alkyl chain	+0.120 (29)	30
	Ethyl oleate/25% aq. ethanol, reversed phase, paper, 25° (D)	phenols	+0.305 (2)	
		—ring attached§	+0.220§§ (2)	59
	Olive oil/70% aq. ethanol, reversed phase, paper (E)	*p*-cresols	+0.134 (2)	66
	System E	phenols, *p*-alkoxyphenols, and their respective benzoates	+0.129 (57)	26
	Silanized silica gel/borate buffer–dioxan (90:10), reversed phase (F)	*p*-hydroxybenzoates	+0.180 (3)	67
	Silicone oil/54% aq. acetone (i) 54% aq. methanol (ii) reversed phase, thin layer (G)	testosterone esters —in ester side-chain	+0.190 (i) (2) +0.170 (ii) (2)	50
	Silicone oil/aqueous acetone, reversed phase, thin layer (H)	N,N′-bis(dichloroacetyldiamines) and substituted naphthoquinones	+0.134 (12)	68
	1-Octanol/water–acetone (9:1), reversed phase, thin layer, 20° (I)	acetanilides —attached to an ether oxygen	+0.102§§ (2)	52
	Cellulose/water, paper, non-reversed, 25° (J)	aliphatic acids —derived in an aliphatic system	+0.27§§ (2)	69
Methyl group	System I (i)	acetanilides	+0.143 (i) (2)	
	Liquid paraffin/water–acetone (8:2) reversed phase, thin layer, 20° (ii) (K)	—*para* attached	+0.077 (ii) (2)	52
	System J	aliphatic acids —attached centrally to aliphatic chain	+0.270 (2)	69
	System F	*p*-hydroxybenzoates —alkyl ester group	+0.42 (2)	67

	polyamide/acetone–water (1:1), reversed phase, thin layer, 24° (L)	phenols —ring substituted	+0.16	77
	Triethylene glycol/isooctane, paper, non-reversed phase (M)	cresols and phenol —(i) 4-CH_3 group —(ii) 3-CH_3 group } no vicinal effects	+0.165 (i) (2) +0.165 (ii) (2)	56
	System D	cresols, xylenols, phenols —(i) *ortho*, (ii) *meta*, (iii) *para*	+0.516§§ (i) (2) +0.296 (ii) (2) +0.296 (iii) (2)	59
	System F, with values extrapolated to a theoretical 100% water composition	penicillins —non-terminal, in an aliphatic side-chain and adjacent to an ether oxygen	+0.14 (2)	42
	1-Octanol/aqueous buffer, reversed phase, thin layer (N)	sulphonamides —attached to a pyrimidine ring —(i) adjacent to a nitrogen atom —(ii) *meta* to the nitrogen atoms	−0.34§§ (2) −0.10§§ (2)	78
	Benzene/formamide impregnated paper, non-reversed phase, 20° (O)	(i) cinnamic acids —ring substituted (*para*)	+0.29 (i) (4)	79
		(ii) β-aryl-*n*-butyric acids	+0.27 (ii) (3)	
Benzyl group	System F	*p*-hydroxybenzoates	+1.21 (2)	67
	System C	alkyldinitrophenols —introduced into an aliphatic chain	+0.362 (6)	30
Phenyl group	System E	*p*-substituted cresols	+0.373 (4)	40
	System O	β-aryl-*n*-butyric acids —ring attached (*para*)	+0.760 (4)	79
	System C	substituted alkyldinitrophenols —aliphatic side-chain	+0.183 (5)	30
Cycloalkyl group	System C	substituted alkyldinitrophenols —group introduced into an aliphatic side-chain:		
		(i) cyclobutyl	+0.337 (i) (5)	30
		(ii) cyclopentyl	+0.423 (ii) (5)	
		(iii) cyclohexyl	+0.441 (iii) (5)	
Hydroxyl group	System L	phenols —(i) *ortho*, (ii) *meta*, (iii) *para*	−0.09§§ (i) (2) −0.20 (ii) (2) −0.34 (iii) (2)	77

(Continued on p. 22)

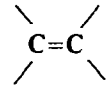
TABLE 2 *(continued)*

Functional group	*Chromatographic system*[*,**]	*Solute and comments concerning the position of the functional group*	$-\Delta R_M$ *value*[***]	*Ref.*
	Ethyl acetate–water–acetone (3:1:1), paper, non-reversed (P)	primary alcohols —(i) primary hydroxyl, (ii) secondary hydroxyl, (iii) tertiary hydroxyl	−0.73 (i) −0.50 (ii) −0.58 (iii)	81
	System M	cresols, phenol —*meta* and *para* hydroxyl	−0.299 (3)	56
	System J	aliphatic acids —alkyl chain substitution	−0.56 (2)	69
	System K	acetanilides —(i) *para*, (ii) *ortho*	−0.216 (i) (2) −0.217[§§] (ii) (2)	52
	System I	acetanilides —(i) *para*, (ii) *ortho*	−0.302 (i) (2) −0.494 (ii) (2)	52
Methoxy group	System (I) (i) System (K) (ii) System (N)	acetanilides —*para* substituted sulphonamides —(i) adjacent to a nitrogen atom in a pyrimidine ring —(ii) *meta* to the pyrimidine nitrogens	−0.114 (i) (2) −0.120 (ii) (2) −0.370[§§] (i) (2) −0.16 (ii) (2)	52 78
	System D	phenols —*para*	−0.007 (2)	59
	System O	(i) cinnamic acids (ii) β-aryl-*n*-butyric acids	+0.080 (8) +0.100 (4)	79
Amino group	System I (i) System K (ii) System M	acetanilides —*para* phenols —(i) *para*, (ii) *meta*	−0.756 (i) (2) −0.502 (ii) (2) −1.762 (i) (2) −1.720 (ii) (2)	52 56
	Ethanol–conc. NH_3–water (80:4:16) (i) *n*-Butanol–acetic acid–water (4:1:5) (ii) System P (iii)	aliphatic acids —α-amino group	−0.24 (i) −0.71 (ii) −1.36 (iii)	81, through ref. 31
	System F, with values extrapolated to a theoretical 100% water composition	penicillins —introduced into aliphatic chain	−0.480 (2)	42
	n-Amyl alcohol/5 *N* formic acid, reversed phase, thin layer (Q)	aliphatic acids —α-amino group in the ionised state	−3.3	41

$-N=CH_2$ group	System (F), at 100% water composition	penicillins —in an aliphatic side-chain	−0.830 (2)	42
Nitro group	System (I) (i) System (K) (ii) System (O) Polyamide gypsum/1-butanol–5 *N* NH_3 (100:33) (R)	acetanilides —*para* cinnamic acids —*ortho*-NO_2, adjacent to a hydroxyl group	+0.453 (i) (2) +0.342 (ii) (2) −0.46 (8) +0.15[55] (2)	52 79 82
Fluoro group	System I (i) System K (ii)	acetanilides —*para*	+0.299 (i) (2) +0.161 (ii) (2)	52
$-CF_3$ group	System O	*β*-aryl-*n*-butyric acids —*meta*	−0.40 (4)	79
	Paraffin oil/acetone–water–dioxan (1:2:1), reversed phase, thin layer (S)	phenylhydrazones —(i) 2-CF_3, (ii) 3-CF_3, (iii) 4-CF_3	+0.149 (i) (2) +0.195 (ii) (2) +0.077 (iii) (2)	60
4-SCF group (i) 4-SO_2CF_3 group (ii)	System S	phenylhydrazones	+0.185 (i) (2) +0.443 (ii) (2)	60
Chloro group	System N	sulphonamides —adjacent to N atom in pyrimidine ring	−0.07 (4)	78
	System I System K	acetanilides —*para*	+0.690 (2) +0.440 (2)	52
	System F, with values at 100% water composition	penicillins —adjacent to a N–O–N conjugated system	+0.290 (2)	42
	Methanol/methanol–water, reversed phase, paper (T)	testosterones —4-chloro	+0.26 (4)	80
	Propylene glycol/toluene, non-reversed phase, paper (U)	testosterones —4-chloro	+0.31 (4)	80
	System M	phenols —*meta* and *para*	+0.165	56
	System O	cinnamic acids —(i) *meta*, (ii) *para* *β*-aryl-*n*-butyric acids —ring-substituted (*para*) (iii)	+0.22 (i) (8) +0.29 (ii) (8) +0.31 (iii) (4)	79
Bromo group	System I (i) System K (ii)	acetanilides —*para*	+0.947 (i) (2) +0.556 (ii) (2)	52

(Continued on p. 24)

TABLE 2 *(continued)*

Functional group	*Chromatographic system**,***	*Solute and comments concerning the position of the functional group*	ΔR_M *value****	*Ref.*
	System O	cinnamic acids —*meta* and *para*	+0.26 (4)	79
	System O	*β*-aryl-*n*-butyric acids —(i) *meta*, (ii) *para*	+0.330 (i) (4) +0.250 (ii) (4)	79
Iodo group	System I (i) System K (ii)	acetanilides —*para*	+1.148 (2) +0.721 (2)	52
	System O	*β*-aryl-*n*-butyric acids —*ortho*	+0.400§§ (2)	79
	System O	cinnamic acids —(i) *meta*, (ii) *para*	+0.400 (i) (2) +0.330 (ii) (2)	79
Formyl group	System I (i) System K (ii)	acetanilides —*para*	+0.075 (i) (2) +0.015 (ii) (2)	52
Carboxyl group§§	System I (i) System K (ii)	acetanilides —*para*	−0.672 (i) (2) −0.694 (ii) (2)	52
	System F, with values at 100% water composition	penicillins —introduced into an aliphatic chain	−1.01 (2)	42
Structural arrangements†	System B	tocyl phenols, etc. (i) isoprene unit (ii) hydrogenated isoprene unit (iii)  (iv) branching (alkyl chain)	 +0.249 +0.366 −0.121 $(n-1) \times \Delta R_M$ value	26

* All ΔR_M values have been given reversed-phase notation.

** Multiple citation of any particular chromatographic system is achieved by quoting the reference letter first given to the system.

*** Arabic numerals in parentheses after the ΔR_M values indicate the number of compounds from which the value was obtained.

§ First-member anomaly (see text).

§§ Effects taking place which should cause non-additivity when using this value.

§§§ Other values are given in the text.

† See also Lien *et al.*[49] for the effect of conformational changes on the R_M values for some thiolactams.

prefix sign for a reversed-phase situation, that is, a high positive ΔR_M value indicates a large hydrophobic character of the function. The table also indicates those values whose derivation is from systems where the previously discussed non-additivity effects may be occurring. Values, where necessary, have been arranged into groups according to the type of solvent pair in which they were determined. Also indicated are the number of compounds from which each individual value has been derived.

The methylene group is perhaps the most largely examined function, mainly because of the ready availability of homologous series of compounds. A mean value for the $\Delta R_{M(CH_2)}$ group of +0.161 can be obtained from the table for cases in which the additivity rule should be obeyed. This represents a value derived from 119 separate structure determinations of R_M. Early studies by Howe[70], and a citation of non-consistency of $\Delta R_{M(CH_2)}$ values with fatty acid dinitrophenylhydrazines on paper chromatograms by Bush[41] were thought to disprove Martin's postulates regarding additivity. Green and McHale[40], however, explain that these effects are due partly to experimental deviations (especially due to $R_F > 0.8$ values), but also to the fact that there is variable but strong adsorption of this functional group onto paper[71]. When an aromatic system is substituted by an aliphatic side-chain and the methylene group incremental values to the R_M change analysed, there can often be exhibited a first-member anomalous value. This anomaly may be due to the chromatographic system in which the solutes are examined, and does not necessarily arise in all systems. For example, the first-member anomaly occurs with alkylbenzoates measured in direct phase systems[72], but it does not occur when measured by reversed-phase methods[39]. Marcinkiewicz *et al.*, in a study of the methylene group value, found that when the group was situated close to the attachment of aliphatic chains to an aromatic nucleus, ΔR_M values went significantly lower when measured in a direct phase system than when measured in a reversed-phase system of low polarity. For example, ethyl oleate–25% aqueous ethanol; for such a system, calculated values of group ΔR_M constants for methylene groups substituted further and further away from the point of attachment are as follows: $\alpha(CH_2) = +0.291$; $\beta(CH_2) = +0.0359$; $\gamma(CH_2) = +0.427$; $\delta(CH_2)$ and ε–$\omega(CH_2) = +0.452$. The effect is, however, not shown in solvent systems of lower water content (*e.g.*, olive oil–70% aqueous ethanol).

Using this latter system, by varying the water content of the mobile aqueous phase, changes can be seen in the $\Delta R_{M(CH_2)}$ value, *viz.*, +0.245 (50% water) content; +0.129 (30%); +0.103 (5%). Similarly, a mean value of +0.455 for the methylene group has been calculated[59] as the homologous incremental value in a series of *p*-alkyl-substituted phenols. In this study a paper reversed-phase technique using ethyl oleate and 25% aqueous ethanol was used. The value obtained is constant only when the methylene group is sufficiently far removed from any functional group which could interact with it.

In recent studies Wawrzynowicz and Santos[73], examining the chromatography of some substituted alkaloids by descending paper partition chromatography found that in moving from an alkaloid –OH to an alkaloid $-OCH_3$ there was no constancy of $\Delta R_{M(CH_2)}$. (Although in further moving to an alkaloid $-OC_2H_5$ molecule a consistent value of between +0.40 to +0.42 was obtained.)

Such findings reinforce the case put forward in Section 4 for citation of R_M values at 100% pure solvent compositions.

Clifford *et al.*[74], in a study on some 2-(1-substituted)-4,6-dinitrophenols,

have given values for the R_M of phenols with up to an *n*-octyl side-chain. Analysis of their data (Fig. 6) shows a deviation from linearity of the *n* *vs.* R_M relationship from about $n = 7$ onwards. Molecular models show that for these compounds a shielding of the phenolic polar grouping can occur by long chains of seven or more alkyl chains, *i.e.* when substitution of the 2-methyl group is by *n*-hexyl or above. Similar shielding effects for the methylene group have been shown by Bark and Graham[75] with 3- and 4-alkyl-substituted phenols.

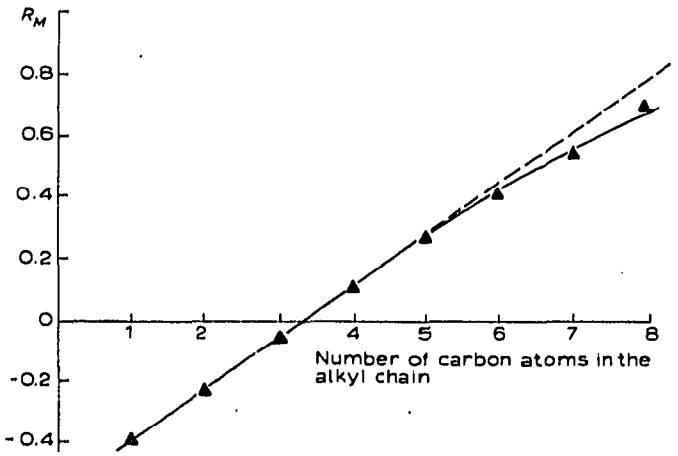


Fig. 6. Relationship between the number of carbon atoms in the alkyl chain of a series of 2-(1-substituted)-4,6-dinitrophenols and their R_M values as measured in a reversed-phase system. (After Clifford *et al.*[74].) Departure from linearity is thought to be due to a shielding effect by the alkyl chain of the phenolic hydroxyl group.

Davis[76], in a study of the thermodynamics of the methyl group in drug molecule solutions, has clearly identified that the free energy of transfer values of the methyl group differ from those of the methylene group, and that the methyl group has a different character, depending upon its position in the molecule. Table 2 shows that ring-attached methyl groups have a mean value of +0.208 when there are no vicinal effects. This is similar but not equal to the $\Delta R_{M(CH_2)}$ value when measured attached or close to an aromatic ring system. The aliphatic methyl group ΔR_M value derived by Layole *et al.*[69] (+0.270) is similar to an aliphatic methylene group ΔR_M value derived in the same system.

Bush[31] has suggested that a modification procedure should be followed when using $\Delta R_{M(CH_2)}$ values for the prediction of unknown molecular R_M values. To this already involved list must be added a modification for the methyl group values when the group is, for example, terminal to an aliphatic side-chain. Although it is possible to follow this modification procedure to calculate R_M values for structures with ring systems, it is recommended that experimentally determined $\Delta R_{M(ring)}$ values are used. Accordingly, Table 2 gives values for phenyl, benzyl and cycloalkyl ring systems. Analysis of the cycloalkyl data shows that the methylene incremental values (of +0.086 and +0.018) in these ring systems are variable and lower than for non-ring methylene values, as has been discussed earlier. The marked differences in aromatic

nucleus attached, and aliphatic side-chain attached values for the phenyl group should be noted; the effect is probably due to hyperconjugation.

Non-additivity of polar groups has been discussed previously and Table 2 shows the effect of ring position and side chain position of the ΔR_M values of a number of polar groupings, for example, hydroxyl, amino. The hydroxyl ΔR_M value found in steroids is very different from the values shown for the hydroxyl group, probably due to the steric effects of the alicyclic rings. Hüttenrauch and Scheffler[80], using reversed and straight chromatographic systems, have given values for the 11β-hydroxy group in testosterone esters of -0.97 and -1.04, respectively. And Bush[41] has stated that in straight-phase chromatography the 11β-hydroxy group in some substituted progesterones has a value of -0.75, becoming -1.15 when measured by a reversed-phase method.

Similarly, Bush also showed that the ΔR_M value for the 14β-hydroxy group in steroids has a value of -1.17, which is similar to the mean value for hindered axial secondary groups such as the 11β-hydroxy group. For a complete listing of characteristic hydroxyl ΔR_M values, for different positions and orientations in steroids, attention is drawn to ref. 41 (p. 87) and ref. 31 (p. 419).

Methoxyl group effects are composed of two opposing forces, the effect of the lone-pair electrons on the oxygen and the inductive effect of the alkyl group. As discussed elsewhere, they are thus greatly dependent upon the chromatographic solvent system in which they are measured. This is true for all similar groups, and examples can be found in Table 2. For example, the value of -1.8 for unionised amino groups should be compared to the values of -3.3 and -2.95 given for this group when it is in the ionized state.

Similarly for carboxyl groups, Bush has given literature values of -0.63 to 0.68 (reversed-phase notation) for the unionized species, which is approximately 1.26–1.48 $\times$ ΔR_M (secondary hydroxyl group)[81].

Literature halogen group ΔR_M values are few. Table 2 lists values found. It can be seen that there is a general trend towards an increase in hydrophobic character of the group as its molar volume increases (that is, as one descends the periodic table). Great difficulty can arise with prediction of R_M values for heterocyclic compounds, because they can exist in aqueous solution in different conformations. Prediction will be uncertain when dealing with possible vicinal effects because interactions may be promoted or hindered by the particular conformational arrangement in which the groups find themselves. An interesting point here, and this can apply to some other structures, is that eventual accurate prediction or even measurement can be made of the R_M value of any conformer, but it is unknown whether in the biologic situation any particular conformational state exists. Conversely, a fall down in any QSAR model upon introduction of a molecule with a determined conformation may well indicate a change in conformation when in the biologic system it is being studied in.

Although the values given in the table are not exhaustive, consideration of the foregoing discussion in this section, and in section three, should enable the medicinal chemist to formulate R_M values for most drug molecules. However, the experiences of many workers have shown that it is far more satisfactory to measure R_M or log P values experimentally, and then to use these values in QSAR models, than it is to use predicted values. As discussed previously, chromatographic methods facilitate this approach.

TABLE 3

REGRESSION EQUATIONS FOR THE RELATIONSHIP BETWEEN HYDROPHOBIC CONSTANTS DERIVED FROM NORMAL PARTITION COEFFICIENT MEASUREMENTS AND THOSE OBTAINED BY CHROMATOGRAPHIC TECHNIQUES

All relationships have been given reversed-phase notation. Log P or π from a 1-octanol system except when indicated otherwise.

Solute	*Stationary phase*	*a*	*b*	*n*	*r*	*s*	*Eqn.*	*Ref.*
(i) $\pi = aR_M + b$								
Heterocyclic ring-substituted sulphonamides*	silicone oil 5%**	0.973	0.485	16	0.961	0.191	5.3	78
	silicone oil 10%***	0.931	0.399	16	0.962	0.189	5.4	78
	silicone oil 20%***	0.901	0.070	16	0.974	0.156	5.5	78
	1-octanol 5%***	1.032	0.663	16	0.925	0.262	5.6	78
	1-octanol 10%***	1.098	0.578	16	0.947	0.221	5.7	78
	1-octanol 20%***	1.091	0.477	16	0.961	0.189	5.8	78
	silicone oil 5%§	0.841	1.144	16	0.666	0.644	5.9	78
	silicone oil 5%§§	0.961	0.754	16	0.796	0.499	5.10	78
	silicone oil 5%§§§	0.967	1.117	16	0.747	0.588	5.11	78
	silicone oil 5%***	0.826	0.254	12	0.975	0.152	5.12	78
	silicone oil 10%***	0.826	0.100	12	0.972	0.159	5.13	78
	silicone oil 20%***	0.846	−0.025	12	0.978	0.142	5.14	78
	liquid paraffin 5%***	0.795	0.055	12	0.979	0.138	5.15	78
	liquid paraffin 10%***	0.774	−0.035	12	0.979	0.137	5.16	78
	liquid paraffin 20%***	0.783	−0.101	12	0.978	0.139	5.17	78
	squalane 5%***	0.765	0.099	12	0.981	0.131	5.18	78
	squalane 10%***	0.778	−0.003	12	0.984	0.121	5.19	78
	undecane 5%***	0.803	0.202	12	0.979	0.136	5.20	78
	undecane 10%***	0.799	0.110	12	0.981	0.129	5.21	78

(ii) $\log P = aR_M + b$								
Cinnamic acid derivatives**	non-reversed phase, benzene/formamide impregnated paper	1.715	−1.747	35	0.984	0.192	5.22	79
Thiolactams	non-reversed phase, silica gel							
	(i) dioxan	7.253	−5.127	5	0.959	0.203	5.23	49
	(ii) butanol–acetic acid–water (4:2:1)	4.053	−2.033	5	0.968	0.180	5.24	49
(iii) $\Delta R_M = a\pi + b$								
Acetanilides and triazinones	polyamide plates	0.456	0.027	42	0.991	0.075	5.25	85
Acetanilides	liquid paraffin	0.561	−0.017	16	0.959	0.165	5.26	53
	1-octanol	0.832	0.022	16	0.987	0.109	5.27	53
Triazinones	polyamide	0.462	−0.001	26	0.936	0.160	5.28	51
(iv) $R_M = a\pi + b$								
Testosterone esters	silicone oil 5%/acetone–water (54:46)	0.288	−0.143	14	0.964	0.119	5.29	50
	silicone oil 5%/methanol–water (54:46)	0.394	0.496	14	0.981	0.118	5.30	50
(v) $R_M = a \log P + b$								
Penicillins**	silicone oil 5%	0.434	−0.225	6	0.892	0.236	5.31	86

* Values of R_M corrected for ionisation.
** Predicted log P values, using $\Sigma\pi$.
*** Isobutanol/water partition coefficient.
§ Chloroform/water partition coefficient.
§§ Toluene/aqueous phase partition coefficient.
§§§ Ethylene chloride/aqueous phase partition coefficient.

The increasing attention being paid to physicochemical parameters as indexes of hydrophobicity has led to various studies correlating different hydrophobic parameters with another. Such studies mainly relate the partition coefficient, or the π substituent constant, to other terms such as polarizability, parachor and molar attraction forces, etc. Leo *et al.*[83] have found that the partition coefficient gave better correlations for several series of compounds than obtained using these afore-mentioned terms. Although this does not necessarily mean that the log P term is a better measure of hydrophobicity *per se*, it does indicate that successful correlations of biological activity with such indices can be interpreted[84] as indications of the importance of the solubility properties of the compounds.

Relatively little work has been published on the relationships that exist between R_M or ΔR_M values measured in thin-layer or paper systems, and other parameters used in QSAR models. The most obvious relationship is that between the partition coefficient and R_M (and/or their respective substituent constants, π and ΔR_M). In Table 3, correlations between these two indices, as reported in the literature, are shown. An average correlation coefficient of 0.944 can be computed for all the values in the table, and although, obviously, these are for mostly successful correlations, the results are added evidence for the validity of the extension of Collander's postulates that partitioning indices from solvent system to solvent system can be correlated.

The most interesting study relating the two indices has been the recent examination by Biagi and his co-workers[78] of the relationships between π and R_M values for some heterocyclic substituted sulphonamides. The study reveals (eqns. 5.12 to 5.21) equivalent good correlation between π values obtained from an isobutanol/water system and R_M values obtained from determinations in three separate reversed-phase chromatographic systems, and measured in each at three different non-aqueous phase concentrations. Similar equivalent good correlations between the two indices, when measured for the same solute in different systems, are shown elsewhere in the table.

Of particular note is the study relating ΔR_M and π values of triazinones[53] to one another. Here, for 26 triazinones, a reasonable correlation of 0.936 is found between ΔR_M values obtained from R_M determinations using commercial polyamide thin-layer plates, and π values from a 1-octanol/water system. Dearden *et al.*[85] have taken these values and included them as a sub-set in an analysis of the relationship between ΔR_M (polyamide) and π (1-octanol) values. Including into the analysis two sub-sets of alkyl-substituted and *para*-substituted acetanilides, they were able to obtain an improved correlation coefficient of 0.991 (eqn. 5.25). It is argued that this is a proof of the general validity of the ΔR_M *vs.* π relationship studied for widely differing classes of compounds. However, although the "unexplained" variance between the data for eqn. 5.28 improves from 12 to 2% in eqn. 5.25, because of the increased number of values used in deriving the regression equation, unless the added values are totally dissimilar an improved correlation is to be expected (see Section 2).

For those relationships showing good correlation, it is a reflection that the two indices are of similar rank order as measures of hydrophobicity. This does not preclude one giving better correlations in QSAR models, as will be shown in the following section.

Eqns. 5.9–5.11, which are taken from the study by Biagi *et al.*, are included in the table as they indicate situations where the modified Collander relationship is not

valid. Using R_M values from one reversed-phase system (silicone oil 5%/aqueous buffer), correlations were of low statistical significance when related to π values obtained from partition coefficient measurements from three systems where the non-aqueous phases were chloroform (eqn. 5.9), toluene (eqn. 5.10), and ethylene dichloride (eqn. 5.11). These show that between 37 to 56% of the variances between π and R_M data are "unexplained" by the relationship ($\pi = aR_M + b$). This is reduced to a value of 8% when π values from an isobutanol system are used (eqn. 5.3).

The regression coefficients for the slopes of the equations in Table 3 indicate whether the free energy of transfer of a solute, or one of its substituents, from an aqueous phase to a non-aqueous phase is similar for transfer in a chromatographic system to the transfer measured by a partition coefficient technique. An exact comparison is not often possible because of the nature, and often change of polarity, of the aqueous phase used in the chromatographic determinations. However, in those systems where the non-aqueous phase is the same in both techniques (for example, 1-octanol in eqn. 5.27), the slope coefficients approach unity, indicating that the free energy of transfer of the solute is similar (but not necessarily equal) in both.

The method of regression analysis of data is also useful in elucidating whether additional parameters need to be used for describing one index in terms of the other. In a study of the effect of ionization on the chromatographic behaviour of some β-aryl-*n*-butyric acids, Kuchař *et al.*[79] have derived equations relating R_M values to π values with R_M values derived from a chromatographic system (A) where the acids would be ionised and a system (B) where they are not. Using literature π values measured in a 1-octanol system, their derived equations (with reversed-phase notation), are

$$\pi = 1.587\,R_M{}^{A} - 0.321; \quad n = 13;\ r = 0.920;\ s = 0.208 \tag{5.32}$$

$$\pi = 1.674\,R_M{}^{A} + 0.603\,\sigma - 0.264; \quad n = 13;\ r = 0.963;\ s = 0.150 \tag{5.33}$$

$$\pi = 1.783\,R_M{}^{B} + 0.090; \quad n = 13;\ r = 0.959;\ s = 0.150 \tag{5.34}$$

In system A, the introduction of the Hammett electronic term σ improves the correlation over the straight π *versus* R_M, whereas the σ term is not needed for system B. These findings could indicate that if R_M determinations are made in systems where the solute is ionised, then for QSAR purposes a ($R_M + \sigma$) term is to be used as an index of hydrophobicity.

The hydrophobic fragmentational constants, f, introduced recently by Nys and Rekker[65] to overcome non-additivity of π in such situations as are found when predicting log P of a structure with an aliphatic alkyl chain having a terminal polar group, are found to correlate well with R_M values. For example, in eqn. 5.29 (Table 3), the relationship derived between partition values gives a correlation of 0.964 (and a variance ratio F value of 157[65]), if however, the f values of Nys and Rekker are used instead of π values, the following relationship is found:

$$R_M = 0.279f - 0.073; \quad n = 14;\ r = 0.980;\ s = 0.086 \tag{5.35}$$

This is a significant improvement ($F = 306$) over eqn. 5.29 and has been attributed to the better correlation of the R_M and f values of the testosterone phenylproprionate ester.

Another mutual correlation which has been found between R_M and another

parameter is the correlation between Zahradnik β constants and R_M constants as noted by Kopecky and Boček[87]. These β constants are regarded as analogous to the π parameter.

6. R_M CORRELATION WITH BIOCHEMICAL AND BIOLOGICAL SYSTEMS

As discussed previously (Introduction), the overwhelming evidence that the lipophilic character of a drug molecule can be of vital importance in the processes affecting the action of a drug, is now well documented[1]. In the studies of Meyer[88] and of Overton[89] it was found that the narcotic potency of the members of a set of congeners tends to increase as their oil–water partition coefficient increases, which aroused interest in the characterization of lipophilicity and its relationship to drug effect. This section is concerned with the use of R_M and ΔR_M chromatographic parameters as extrathermodynamic parameters when used in QSAR models of the LFER type. These parameters have found application both with *in vivo* and *in vitro* systems.

For the purposes of this particular study two distinct QSAR models are identified. One, which will be discussed first, involving a rectilinear relationship between the hydrophobic index and activity, and the other, involving a non-linear and sometimes parabolic effect. For comparison purposes in the following discussion, the statistical correlation between activity and log P or π as the index of hydrophobicity is shown. For the biochemical or pharmacological significance of the derived relationships shown, the reader is advised to consult the appropriate literature reference.

A. Linear relationships between R_M, ΔR_M and activity

It is possible[90] for the partition coefficient of a drug in a biochemical system to be defined as

$$P_{(bio)} = \frac{C_{(bio)}}{C_{(water)}} \tag{6.1}$$

where $C_{(bio)}$ and $C_{(water)}$ are the molar concentrations of drug in the biophase and in the aqueous phase, when the biophase can be protein, lipid etc. A similar relationship can be assumed for a biological system where $C_{(bio)}$ and $C_{(water)}$ now refer to the non-polar and polar "biophases" of the system. Following on from the considerations given to Collander's work in preceding sections and assuming the R_M parameter to be the index of partition or hydrophobicity, it is possible to write

$$\log P_{(bio)} = a\, R_{M(exp)} + b \tag{6.2}$$

where $R_{M(exp)}$ is the experimentally determined R_M value and $\log P_{(bio)}$ has the same definition as before, and refers to the partitioning of a drug between the aqueous phase adjacent to the critical biophase in which it has its effect. It is on the extra-thermodynamic relationship provided by eqn. 6.2 that the rectilinear dependence of drug action on hydrophobic character is based. Hansch and Dunn[90] have shown how eqn. 6.2 can be related to a linear free-energy model describing drug concentration,

at or near equilibrium, at a receptor site. Their treatment of the physico-chemical description of drug effect in biophase systems is outside the scope of this study; it is possible, however, to replace log P in their derivation by the R_M term and produce a modified relationship for their model of linear dependence of drug action on hydrophobic character, that is

$$\log \frac{1}{C} = aR_{M(exp)} + \text{constant} \qquad (6.3)$$

where C is the equivalent molar concentration of a series of drugs producing an equivalent biological or biochemical effect.

(a) Binding of drugs to proteins

R_M and ΔR_M values have been used with success in characterising the binding of relatively non-polar series of drug molecules to serum albumin. The regression equations derived as shown in Table 4 enable one to see that it is the hydrophobic character of these drugs which determines the extent to which they are bound.

TABLE 4

RELATIONSHIPS BETWEEN DRUG PROTEIN-BINDING PARAMETERS AND CHROMATOGRAPHIC HYDROPHOBIC PARAMETERS

k is the intrinsic association constant for the binding, and BF is a measure of the extent of binding in percentage terms. Eqns. 6.4–6.6 are for ΔR_M values derived in a 1-octanol/acetone–water (1:9) system, eqns. 6.7–6.9 are for a liquid paraffin/acetone–water (2:8) system, and eqn. 6.10 uses R_M values determined in a polyamide/acetone–water–dioxan (1:2:1) system.

Model studied	*a*	*b*	*n*	*r*	*s*	*Eqn.*	*Ref.*
Log $k = a.\Delta R_M + b$							
Acetanilides to bovine serum albumin	0.63	4.38	13	0.981	0.59	6.4	53
	[$\pi_{(1\text{-octanol})}$:		13	0.989	0.37]		
	0.62	4.38	16	0.981	0.65	6.5	53
	[$\pi_{(1\text{-octanol})}$:		16	0.985	0.54]		
	0.70	4.33	18	0.919	0.41	6.6	53
	[$\pi_{(1\text{-octanol})}$:		18	0.887	0.57]		
	0.86	4.22	13	0.943	0.18	6.7	53
	0.87	4.41	16	0.947	0.18	6.8	53
	0.89	4.34	18	0.798	0.97	6.9	53
	0.94	4.36	12	0.981	0.08	6.10	85
	[$\pi_{(1\text{-octanol})}$:		12	0.925	0.15]		
Log $BF = a R_M + b$							
Corticosteroids to serum albumin	0.67	−2.29	9	0.964	0.09	6.11	91

In addition to the shown relationships, Biagi[92], in a study of the lipid solubility and human serum binding of various penicillins, has experimentally shown that for some of the penicillins the correlation between the partition index R_M and human serum binding was greater than that using $\Sigma\pi$ values. Improved correlation was found to be particularly so in the case of benzylpenicillin for which the experimentally deter-

mined R_M value (measured in a reversed-phase silicone oil system) indicated a lipid solubility less than that implicated by the $\Sigma\pi$ calculation.

For purposes of elucidating structural effects on the binding, four regression equations for the binding of acetanilides have been derived[53]: when $n = 13$ the data set is comprised of a series of *p*-substituted compounds, when $n = 12$ the –COOH substituted molecule is excluded from the set, when $n = 16$ N-methylated acetanilides are added to the $n = 13$ set, and finally, when $n = 18$ two compounds, *viz. ortho*-OH and *ortho*-OEt, are included. Eqns. 6.4 to 6.9 in Table 5 show that correlations of reduced significance are found between log k and ΔR_M values measured in a reversed-phase liquid paraffin/acetone–water (2:8) system when compared to those between log k and $\Delta R_{M(1\text{-octanol})}$ and $\pi_{(1\text{-octanol})}$. Correlations between ΔR_M values from a polyamide/acetone–water–dioxan (1:2:1) system and log k (eqn. 6.10) are significantly better than the correlation using $\pi_{(1\text{-octanol})}$ values. The improved correlations found using the 1-octanol and polyamide systems compared to the liquid paraffin system values indicate that the free energy change in binding to bovine serum albumin is similar to the free energy change in transfer from the aqueous phase to the 1-octanol or polyamide phases. As polyamide can be considered as "protein-like" in composition, this may explain the improved correlation found. Clearly, the use of thin-layer polyamide plates for measuring R_M values is an advantageous one for certain situations and must be given consideration by future workers. The slightly polar nature of the alkanol 1-octanol is thought to be reflected in the breakdown in correlation for $n = 18$ (eqn. 6.9) in the log $k/\Delta R_{(\text{liquid paraffin})}$ relationship, *i.e.* when *ortho* groups are included in the data set. For $n = 18$ in the log $k/\Delta R_{M(1\text{-octanol})}$ relation the model still gives reasonable correlation, due perhaps to some competition with the aqueous phase for the acetamido group of the acetanilide by the alkanol hydroxyl. This may produce an increased "hydrophobicity" index, and may be analogous to the situation when drug moves from the aqueous phase to a somewhat polar protein "phase".

(b) Anabolic activity

Chaudry and James[93], using the R_M values of some nandrolone esters obtained from the chromatographic measurements of Hüttenrauch and Scheffler[80], have related the hydrophobicity of these compounds to their anabolic activities measured in the whole animal. Their reported relationship is shown by eqn. 6.12 with reversed-phase notation.

$$\log BR = -0.84\,R_M - 2.35;\ n = 7;\ r = 0.841;\ s = 0.284 \qquad (6.12)$$
$$[\log P\ (\text{ethyl oleate}):\ n = 8;\ r = 0.889;\ s = 0.244]$$

where BR is a function of the biological response produced. The derived expression uses R_M from a straight system using chloroform–water–methanol as the mobile phase. The model was not improved by the introduction of an R_M squared term (see later), and although it has a lower coefficient of correlation than a relationship derived with log P as the index, a direct comparison of the correlation coefficients of the two is not possible because one less compound was used in deriving the equation using R_M. An improved correlation using R_M values can be argued on the basis of variance ratio (F) tests. That is, for the R_M relationship, $F_{1,5} = 80.07$ [α (0.001) = 47.18], and for the log P expression, $F_{1,6} = 18.98$ [α (0.01) = 13.74].

A further example of the relationship between R_M values and steroid ester activity is that derived for the effect of some testosterone esters in the capon's comb test[94], *i.e.*

$$\log BR = 0.416\ R_M + 0.295;\ n = 7;\ r = 0.934;\ s = 0.09 \qquad (6.13)$$

Here, R_M values were measured in a thin-layer reversed-phase system using silicone oil/water–acetone (46:54) as the solvent pair.

(c) Microbiological activity

Linear and non-linear dependence of anti-microbial activity on hydrophobic character of drugs is well known. There is, however, only a single instance of R_M values being well correlated linearly with such activity. This is from the study by Biagi *et al.*[95] of the influence of hydrophobic character on the anti-bacterial activities of some penicillins and cephalosporins. The attempted correlations of the activities of the two drug series, against a number of organisms, and R_M values, generally gave poor correlations when a rectilinear model was used. However, for penicillins against *Escherichia coli* the model was found to be reasonable, *i.e.*

$$\log \frac{1}{C} = -1.304\ R_M + 2.551;\ n = 11;\ r = 0.899;\ s = 0.463 \qquad (6.14)$$

The unexpected negative sign for the slope coefficient indicates that the activity increases with a decrease in hydrophobic character of the penicillins. This indicates that either the *E. coli* cell wall is non-lipid in nature, which is not borne out by other measurements, or the penicillins increasingly tend to remain firmly attached to the first lipid barriers encountered and do not move to their effective site of action.

(d) Absorption and excretion of drugs

The classical experiments of Meyer and Overton have laid the foundations for the pH partition hypothesis of drug absorption from the gastro-intestinal tract. Because of the high protein and lipid content of mucosal membranes, many attempts have been made to correlate drug absorption data with some hydrophobic index. This has usually been the partition coefficient. Plá-Delfina *et al.*[34] have recently found that for a group of barbituric acids studied, if the amount of drug absorbed is correlated with the hydrophobic index, then the rate of absorption is also well correlated. Using well documented gastric absorption data for several barbituric acid derivatives, they have correlated literature R_M values obtained from seven paper chromatographic systems, with their *in vivo* gastric absorption rate constants (k). A summary of their findings is given in Table 5.

Apparently because of the similarity of the pK_a values of the barbituric acid derivatives, no electronic parameter has been included in the independent variable data set, even though between 7 and 25% of the variance between the data is unexplained by the given relationships. As has been previously discussed (see Section 5, eqn. 5.32 and 5.33), the acidic composition of the chromatographic system will affect the correlation of R_M with other hydrophobic indexes if ionisation of the solute is possible. From Table 5, system 5 is seen to give the best correlation of the data. This system may be regarded as an acidic environment. As is also suggested in Section 5,

a R_M + σ term could perhaps have had a use in this particular study. In systems 1 and 7, adsorption effects should be fairly important in the migration of the solute, however reasonable correlations are still obtained between R_M values from these systems and the biological data. Eqn. 6.18 employs R_M values obtained from an anhydrous chromatographic system, illustrating that non-aqueous polar phases can be used instead of water to obtain the partition index, though it is a nice point to state that this is still a "hydrophobic" index.

TABLE 5

REGRESSION EQUATIONS FOR THE RELATIONSHIP $\log k = a R_M + b$, WHERE k IS THE *in vivo* GASTRIC ABSORPTION RATE FOR A SERIES OF BARBITURIC ACID DERIVATIVES

R_M values with reversed-phase notation. Equations derived using R_M values from systems 1, 4 and 5 are significant at the α (0.01) level, the others at the α (0.1) level only. (After Plá-Delfina *et al.*[34].)

Paper chromatographic system	*a*	*b*	*n*	*r*	*Eqn.*
1. Dichloromethane on paper impregnated with 1% Na_3PO_4 in water	0.240	0.681	11	0.865	6.15
2. $CHCl_3$–benzene–5 *N* NH_4OH (13:3:6) on formamide-impregnated paper	0.282	0.748	8	0.872	6.16
3. $CHCl_3$–benzene–formamide–5 *N* NaOH (12:2:1:5) on formamide-impregnated paper	0.374	0.645	8	0.875	6.17
4. Formamide–saturated $CHCl_3$ on formamide-impregnated paper	0.404	0.767	9	0.912	6.18
5. Toluene–acetic acid–water (10:5:4)	0.525	0.993	7	0.967	6.19
6. $CHCl_3$–10% NaOH (10:5)	0.261	0.724	7	0.918	6.20
7. $CHCl_3$–isopropanol–25% NH_4OH (45:45:10)	0.810	0.963	8	0.900	6.21

An examination of the possible use of drug buccal absorption data in man, as an *in vivo* index of hydrophobicity[96] has led Dearden and Tomlinson to examine the correlations between human buccal absorption data of some acetanilide drugs, and their ΔR_M and π substituent values. The found relationships are as follows

$$PA = 28.42\,\Delta R_M^{\wedge} + 26.47;\ n = 18;\ r = 0.986 \qquad (6.22)$$

$$PA = 40.86\,\Delta R_M^{\mathrm{B}} + 27.36;\ n = 18;\ r = 0.965 \qquad (6.23)$$

$$[\pi_{(1\text{-octanol})};\ n = 18;\ r = 0.976]$$

where $\Delta R_M^{\wedge}$ and ΔR_M^{B} refer to substituent constants derived from R_M measurements in a thin-layer system using (A) 1-octanol and (B) liquid paraffin as the non-aqueous phases, and *PA* refers to the percentage drug absorbed in a given test period. Both relationships are significant at the α(0.001) level. The 1-octanol/water solvent pair, compared to the liquid paraffin system, acts as a better model reference system. For 1-octanol, improved correlation of the data is obtained using the chromatographically generated data over that using π values. Similar improvement in correlation has been shown for protein-binding studies (see before).

Improvement in correlation by the use of chromatographic parameters is not always the case. Biliary excretion of penicillins in the rat is better correlated with log

P values (measured in a 1-octanol/water system) than with R_M data measured in a reversed-phase silicone oil/water chromatographic system[97], that is

$$\log PE = -20.84\,R_M + 39.71;\ n = 8;\ r = 0.84 \qquad (6.24)$$

$$[\log P_{(1\text{-octanol})}:\ n = 8;\ r = 0.87]$$

where PE is the percentage of administered drug excreted into the bile. The negative sign of the slope regression coefficient indicates that less drug is excreted as its lipid solubility increases. Both correlation coefficients are low, however, reflecting that perhaps processes other than simple elimination into the bile occur and that an incorrect QSAR model hfis been employed. Such a consideration may invalidate comparison of R_M and log P usage in this example.

(e) Toxicity

The acute lethal toxicities in mice of five thiolactam compounds have been better correlated with their R_M values as measured in two chromatographic systems than with their log $P_{(1\text{-octanol})}$ values[49] (eqns. 6.25 and 6.26, showing reversed-phase notation)

$$\log \frac{1}{C} = 7.571 R_{M(\text{dioxan})} - 2.850;\ n = 5;\ r = 0.955;\ s = 0.193 \qquad (6.25)$$

$$\log \frac{1}{C} = 4.460 R_{M(\text{BAW})} - 0.185;\ n = 5;\ r = 0.944;\ s = 0.243 \qquad (6.26)$$

$$[\log P_{(1\text{-octanol})};\ n = 5;\ r = 0.929;\ s = 0.272]$$

where $R_{M(\text{dioxan})}$ and $R_{M(\text{BAW})}$ are the R_M values for the thiolactams measured using silica gel on Baker flex sheets as the stationary phase and dioxan and butanol–acetic acid–water (4:2:1) as the two mobile phases, respectively. C is the molar drug concentration producing an equivalent lethal effect in the mice.

B. Non-linear relationships between R_M, ΔR_M and activity

In some early studies[98] on the relationships between structure and activity, it was common to find an initial rectilinear relationship between activity and lipophilicity, followed by a non-linear effect which was termed the "cut-off" point. Over the last decade, Hansch and his co-workers have collected a large number of examples of such relationships showing this departure from rectilinearity, and have accumulated a large amount of evidence which clearly demonstrate that the change to non-linearity is not a sharp one. This leads them to conclude that the term "cut-off" is not well suited to describe the phenomenon. In fact, they have shown that a parabolic, or quadratic expression, is one which appears to fit the data best. Using the R_M term as the index of hydrophobicity, this expression can be written as

$$\log \frac{1}{C} = -a(R_M)^2 + b(R_M) + c \qquad (6.27)$$

where C is the molar concentration of drug producing a standard response in a constant time. Hansch and Fujita[14] have demonstrated that eqn. 6.27 is theoretically related to the probablistic movement of a drug from an extracellular phase to its site of action (assuming normal Gaussian-type distribution of the drug). The use of R_M and ΔR_M values in such a QSAR model is now discussed.

(a) Toxicity

The earliest reported study relating the R_M parameter to a biological activity was that by Boyce and Milborrow[7], who correlated the molluscicidal activities of some N-*n*-alkyltritylamines with their R_M values obtained from thin-layer reversed-phase measurements, using 5% liquid paraffin as the impregnated stationary phase, and acetone–water (7:3) as the mobile phase. A parabolic relation was obtained, and although results were expressed in the graphical form only, the optimum activity of the N-*n*-alkyltritylamines was found for those compounds having an R_M value $\approx +0.1$. In an attempt to mimic the biological environment more closely, these same workers incorporated casein into the stationary phase, but no change in R_F value was demonstrated.

Using the preferred method of extrapolating R_M values to a theoretical 100% water mobile phase, Biagi[99] has found a quadratic relationship between the logarithm of the reciprocal of the minimum lethal dose in cats, for some cardiac glycosides, and the extrapolated values. Chromatographic measurements were carried out in a thin-layer reversed-phase system using silicone oil and acetone–water mixtures as the two phases. Prior experimentation on the acetone composition *vs.* R_M relationship, using 8 to 48% acetone composition ranges, enabled extrapolation of the R_M values to 100% water composition to be achieved. An R_M value of about +1.8 seems necessary for the cardiac glycosides to exhibit an optimal activity in the test.

(b) Steroidal activity

The relation between lipophilic character and *in vitro* haemolytic activity of a series of testosterone esters using R_M constants provides a means of comparison between such a correlation and that found with $\Sigma\pi$ constants[50].

$$\log BR = 1.502 + 1.561\, R_{M(\text{acetone})} - 1.723(R_M)^2_{(\text{acetone})};\ n = 14;\ R = 0.954;\ s = 0.173 \qquad (6.28)$$

$$\log BR = 0.087 + 2.716 R_{M(\text{methanol})} - 1.020(R_M)^2_{(\text{methanol})};\ n = 14;\ R = 0.949;\ s = 0.189 \qquad (6.29)$$

$$[\Sigma\pi_{(1\text{-octanol})};\ n = 14;\ R = 0.944;\ s = 0.189]$$

The relevant equations are given above, where R now is the multiple correlation coefficient. R_M values were measured in a thin-layer reversed-phase system, using 5% silicone oil as the impregnated stationary phase, and acetone–water or methanol–water mixtures as the mobile phases. From R_M *vs.* % acetone, and R_M *vs.* % methanol composition relationships found experimentally those R_M values corresponding to 54% concentrations of acetone or methanol in the mobile phases were used in the regression analyses. Apparently this has been a subjective choice of

composition, though extrapolation to a 100% water composition R_M values would have been more desirable. Results indicate slight improvement in correlation when R_M parameters are used compared to employment of $\Sigma\pi$ parameters, and again the measured index is better than the predicted one.

(c) Inhibition of mitochondrial electron transport

Parabolic relationships have been found between the R_M values of four homologous series of N,N'-bis(dichloroacetyl)diamines, and one homologous series of substituted naphthoquinones, and their activity in inhibiting *in vitro* mitochondrial electron transport[68]. R_M values were obtained from a thin-layer reversed-phase system using 5% silicone oil as the impregnated stationary phase, and acetone–water mixtures as the mobile phases. Linear relationships were found between percentage acetone composition and R_M, and then used to derive R_M values for a 50% aqueous acetone mobile phase, which were then employed in the regression analysis. Precise statistical analysis of the *in vitro* data and the R_M values enabled separation of the different biochemical effects of the five groups of compounds to be made. Eqn. 6.30 is the derived expression between the R_M values and the *in vitro* activities for all five series of compounds.

$$\log \frac{1}{C} = 4.910 + 1.559R_M - 2.082(R_M)^2;\ n = 26;\ \bar{e} = 0.405 \qquad (6.30)$$

where $\bar{e}$ is the square root of the error mean square, and where the $(R_M)^2$ term is significant at the $\alpha(0.01)$ level. Improved correlation is obtained when each compound series is analysed separately.

(d) Microbiological activity

R_M data have been useful on a number of occasions in relating the antibacterial activities of drugs with their hydrophobic nature. Derived regression equations for the activities of various classes of compounds against some species of bacteria are given in Table 6.

TABLE 6

REGRESSION EQUATIONS FOR DRUG ACTIVITIES AGAINST VARIOUS BACTERIA

Equations are of the general type $\log(1/C) = a(R_M)^2 + bR_M + c$. Barbaro *et al.*[101] have found that for rifamycins no qualitative difference in the quadratic expression between R_M values and antibiotic activity against *S. aureus* exists when studied in liquid and in solid media, indicating that diffusion rates into solid media do not affect the QSAR model. For equations 6.32 and 6.35, R_M values were obtained from a 5% silicone oil/50% aqueous acetone system. R_M values for the remaining equations were from a 5% silicone oil/100% water system (and were calculated from derived percentage acetone composition *vs.* R_M relationships). R = The multiple correlation coefficient.

Bacterium	*Drug*	*a*	*b*	*c*	*n*	*R*	s	*Eqn.*	*Ref.*
E. coli	cephalosporins	−1.113	0.483	2.189	14	0.853	0.416	6.31	95
	rifamycins	−1.608	−0.680	2.020	8	0.947	0.389	6.32	100
S. aureus	cephalosporins	−1.017	2.044	3.566	14	0.919	0.419	6.33	95
	penicillins	−1.537	1.644	4.454	8	0.881	0.344	6.34	95
	rifamycins	−0.508	−0.053	6.382	8	0.886	0.245	6.35	100
T. pallidum	cephalosporins	−1.084	1.637	3.964	14	0.925	0.298	6.36	95
	penicillins	−1.072	0.732	5.567	8	0.847	0.270	6.37	95

Although the R_M and anti-bacterial data was available for eleven penicillins, eqns. 6.34 and 6.36 have been derived for eight compounds only, compounds excluded being methicillin, cloxacillin, and dicloxacillin. Inclusion of these three compounds into the data sets causes deviations in the regression which are thought to be due to the presence of *ortho* substituents on the aromatic rings of the penicillin side-chains. Inclusion of an electronic term into the analyses should produce improved correlation here.

A further study by Biagi *et al.*[102] on the influence of lipophilic character on the biological activity of some oligosaccharide antibiotics, for example neomycin B, has demonstrated the effectiveness of using R_M in this type of correlation.

(e) Fungicidal and herbicidal activity

Those members of the scientific community concerned with plant and crop protection have not been slow in using LFER models for analysis of found structure activity relationships. Clifford *et al.*[30], in analysing the structural requirements for compounds active against the mildew fungus *Podosphaera leucotricha*, have examined by regression analysis the relationship between the fungicidal activities shown by a series of alkyldinitrophenols, and their substituent ΔR_M values. R_M values were measured on cellulose layers impregnated with 10% ethyl oleate and developed with 60% aqueous ethanol as the mobile phase. A quadratic relationship was found for a 4-(1-cyclopentyl-*n*-alkyl)-2,6-dinitrophenol series.

$$\log BR = 7.583 - 11.815\,\Delta R_M + 6.434\,(\Delta R_M)^2 \qquad (6.38)$$

Seven compounds were used to derive the shown expression, which was stated to be "significant" by the authors.

In a study on the herbicidal activities of some triazinones, Draber *et al.*[51] have shown that their substituent ΔR_M constants, obtained from R_M values measured in a reversed-phase TLC system with paraffin oil and water–dioxan mixtures as the chromatographic solvent pair, together with their σ values, are well correlated with their action in inhibiting electron transport in isolated chloroplasts.

(f) Analgesic activity

An improvement in correlation using chromatographic parameters can also be demonstrated for an *in vivo* activity. Dearden and Tomlinson[52] have measured the analgesic potencies of a series of *p*-substituted acetanilides in mice, and have correlated the found results with two groups of ΔR_M values. The derived regression equations are given below

$$\log \frac{1}{C} = -0.911(\Delta R_M{}^{\wedge})^2 + 0.507(\Delta R_M{}^{\wedge}) + 0.452;\ n = 13;\ R = 0.956;\ s = 0.127 \qquad (6.39)$$

$$\log \frac{1}{C} = -1.574(\Delta R_M{}^{\mathrm{II}})^2 + 0.388(\Delta R_M{}^{\mathrm{II}}) + 0.488;\ n = 13;\ R = 0.914;\ s = 0.241 \qquad (6.40)$$

$$[\pi_{(1\text{-octanol})}:\ n = 13;\ R = 0.862;\ s = 0.375]$$

where A and B refer to the chromatographic system when 1-octanol (A) and liquid paraffin (B) are the impregnated non-aqueous phases. Significant improvement in the correlations using ΔR_M constants is further shown by variance ratio tests, which place the significance of eqn. 6.39 at the $\alpha(0.001)$ level, eqn. 6.40 at the $\alpha(0.01)$ level, and the correlation using π, at only the $\alpha(0.1)$ level.

The relation between the chromatographic substituent constants and analgesic potencies is represented graphically by Fig. 7, though a larger series of compounds is shown.

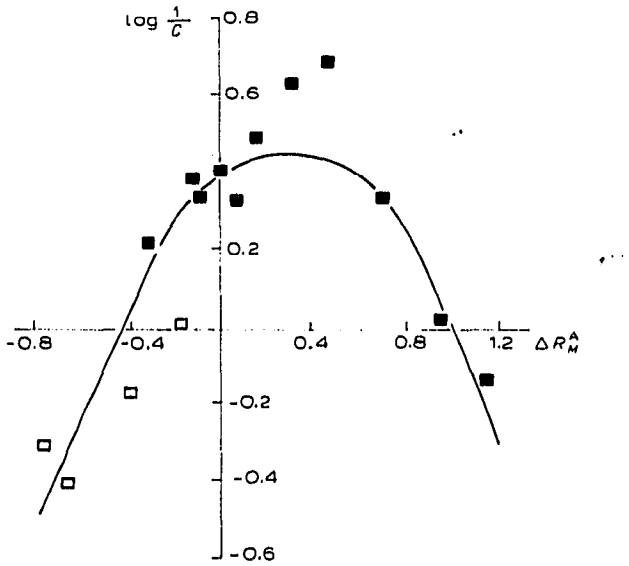


Fig. 7. Relationship between analgesic potency and the substituent constants $\Delta R_M^{\wedge}$ for a series of *para* (■) and *ortho* (□) substituted acetanilides. The drawn curve has been generated from the regression equation found for the relationship (see text).

7. THE $R_{M(opt)}$ AND $\Delta R_{M(opt)}$ PARAMETERS IN DRUG DESIGN

Now that analysis of structure activity relationships by regression techniques is a common one, there exists an ever accumulating amount of data. How may these data be used in the design of new drug candidates? The equation for the LFER relationship, be it rectilinear or quadratic, should be able to provide information on (a) the biological or biochemical processes causing the measured effect and (b) the activity of a chemical structure for which only its physico-chemical description is known. For quadratic relationships, some use has been made of the value of the hydrophobic index at which optimal biological or biochemical results may be achieved within a series of compounds. The value is readily obtained from the regression by obtaining the partial differential of the equation and putting it equal to zero with respect to the hydrophobic parameter, that is

$$\frac{\partial \log \frac{1}{C}}{\partial R_M} = 0$$

Table 7 shows $R_{M(opt)}$ and $\Delta R_{M(opt)}$ values calculated from the regression equations given in Section 6.

These values, at this moment, are of little practical value for they have been obtained from numerous chromatographic systems and no extrapolation of these values to theoretical values in any standardised system is yet possible. Hopefully this will be achieved in the near future.

TABLE 7

$R_{M(opt)}$ AND $\Delta R_{M(opt)}$ PARAMETERS FOR DIFFERENT DRUG TYPES

Drug type	*Chromatographic system*	$R_{M(opt)}$	*Ref.*
N-*n*-Tritylamines (molluscicidal activity)	liquid paraffin/acetone–water (7:3)	0.1	7
	liquid paraffin/100% water phase	4.8 approx.	
Cardiac glycosides (toxicity in cat)	silicone oil/water	1.8	
Testosterone esters (haemolytic activity)	silicone oil/54% aqueous acetone	0.45	50
	silicone oil/54% aqueous methanol	1.55	
N,N'-Bis-(dichloroacetyl-amines)	silicone oil/50% aqueous acetone	0.23	68
Naphthoquinones (inhibition of mitochondrial electron transport)	silicone oil/50% aqueous acetone	0.54	
Cephalosporins	silicone oil/100% water phase		
vs. (i) *E. coli*		0.22	95
(ii) *S. aureus*		1.01	
(iii) *T. pallidum*		0.76	
Rifamycins	silicone oil/50% aqueous acetone	−0.211 (i)	100
		−0.052 (ii)	
Penicillins	silicone oil/100% water phase	0.34	
Dinitrophenols (against mildew fungus)	10% ethyl oleate/60% aqueous ethanol	−0.92*	30
Acetanilides (analgesic activity)	1-octanol/acetone–water (1:9)	0.28*	53
	liquid paraffin/80% aqueous acetone	0.12*	

* Values for the $\Delta R_{M(opt)}$ parameter.

The variation of the $R_{M(opt)}$ parameter with even a change in the polarity of the mobile phase is well illustrated by the values for the N-*n*-tritylamines and their molluscicidal activity. Here, an $R_{M(opt)}$ value of 0.1 is found for acetone–water (7:3). Extrapolation of their data to a 100% water composition now gives a $R_{M(opt)}$ value of about 4.8.

8. CONCLUDING REMARKS

R_M and ΔR_M values have an obvious use in quantitative structure–activity relationships. The weaknesses in their use, as pointed out in the preceding sections,

should not be overlooked, and considerable effort in rectifying the situation would be beneficial to medicinal chemists and physical/organic chemists alike.

The data as discussed in this study ably demonstrate that it is preferable that the hydrophobic index be a measured one, and that the use of $\Sigma\Delta R_M$ is feasible only when either vicinal effects are not present in the studied structure or if ΔR_M values can be obtained from situations which are thought to mimic these vicinal effects..

Following on from Collander's study, and the later studies by Hansch and his co-workers, it should be seen that it is possible to measure R_M values in one chromatographic system and relate them to values obtained in another. If any one chromatographic system can be decided upon as the standard one for this type of study, then this relationship will be of use in obtaining standard R_M and ΔR_M values for use in QSAR models. These relationships between R_M values measured in various systems will be linear if the primary solvation forces in the two solvent systems are alike, so that a range of solutes can be proportionally correlated.

Leo and Hansch have argued that 1-octanol provides an unusually favourable environment by offering both donor and acceptor capability to the hydro- and lipophilic moieties of a compound. However, Rytting *et al.*[103] have suggested that inert hydrocarbons, such as hexane and isooctane, would be more suitable because of the known self-association of alkanols such as 1-octanol, and also because of the fairly high water solubility of water in alkanols. Davis *et al.*[27] have further demonstrated that the free energy of transfer of the methylene group from water to an organic solvent can be considered independent of the solvent, providing this is non-polar in nature. Certainly, if there are no practical difficulties involved, it would appear preferable to use these inert organic solvents in the chromatographic method rather than other "active" solvents.

It is clear that thin-layer and paper chromatographic methods provide a rapid and reproducible technique for obtaining an index of the hydrophobic character of many drugs, an index which further appears to correlate better with biological and biochemical data than the log P parameter. There is no exact theoretical reason why this should be so, though is it possible that the chromatographic process, being a dynamic one producing a parameter derived from a non-steady state function, is more analogous to the biological state than those parameters derived from steady state measurements?

9. SUMMARY

The use of R_M and ΔR_M parameters as indices of hydrophobicity for inclusion in quantitative structure activity relationships has been studied. The relationship between these parameters and other free-energy related parameters is illustrated theoretically and experimentally. It is suggested that the chromatographically obtained parameters could find a wider applicability in structure–activity relationships, and that their use would result in improved correlation of data.

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