VOL. 113 NO. 1 MARCH 12, 1975

NAL OF
HROMATOGRAPHY
RNATIONAL JOURNAL ON CHROMATOGRAPHY, ELECTROPHORESIS AND RELATED METHODS

CHROMATOGRAPHIC REVIEWS (Vol. 19, No. 1)

edited by

Michael Lederer

Please note that Volume 113 (3 issues) will appear at various times in the course of 1975. For publication schedule, see page 3 of the cover.

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JOURNAL OF CHROMATOGRAPHY

VOL. 113 (1975)

JOURNAL
of
CHROMATOGRAPHY

INTERNATIONAL JOURNAL ON CHROMATOGRAPHY,
ELECTROPHORESIS AND RELATED METHODS

VOL. 113 1975

CHROMATOGRAPHIC REVIEWS

(Vol. 19, 1975)

Edited by

MICHAEL LEDERER

(Rome)

Journal of Chromatography, 113 (1975) 1–45
Chromatographic Reviews

CHREV. 85

CHROMATOGRAPHIC HYDROPHOBIC PARAMETERS IN CORRELATION ANALYSIS OF STRUCTURE–ACTIVITY RELATIONSHIPS

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(Received November 26th, 1974)

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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.

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

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

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

where μ_i^{θ} 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^{θ} 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:

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

Eqn. 3.3 shows that the chemical potential based upon mole fractions, is larger than that based upon the molar concentrations, by $RT \ln \overline{V}_s^{0}$, where $\overline{V}_s^{0}$ 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

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

and

$$\mu_T(o) = \mu_L^{\theta}(o) + n\,\mu_H^{\theta}(o) + RT \ln \overline{V}^0(o) + RT \ln C(o) \tag{3.7}$$

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

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

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

$$\Delta\mu^{\theta} = \mu^{\theta}(o) - \mu^{\theta}(w)$$

the following expression is obtained

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

For eqn. 3.9 to be valid a plot of $\log P$ *vs.* n should be a straight line with a slope equal to $\Delta\mu_H^{\theta}/2.303RT$. 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

$$\log P = 0.422\,n - 3.836 \tag{3.10}$$

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,

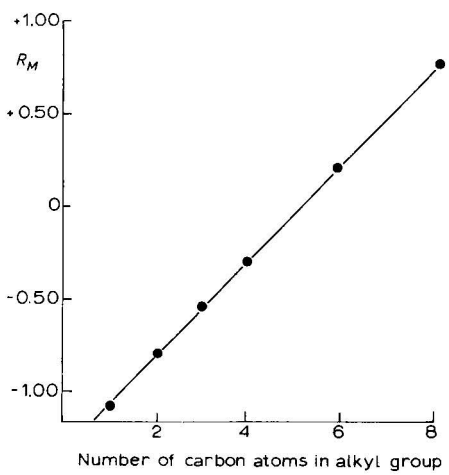


Fig. 1. Relationship between the R_M values and the number of carbon atoms of a series of *n*-alkyl dinitrobenzoates. (After Green *et al.*[26].)

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.

$$\ln P = \frac{\Delta\mu_A}{RT} \tag{3.11}$$

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

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

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

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

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

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

Bate-Smith and Westall[29] introduced the term

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

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

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

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-

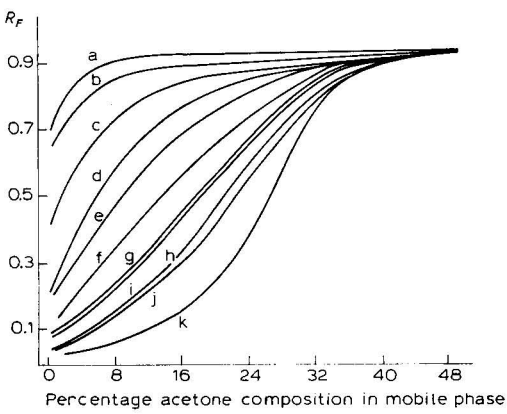


Fig. 2. Experimental curves for the relationship between R_F values and percentage acetone composition in the mobile phase for some penicillins studied using reversed-phase chromatography. (After Biagi *et al.*[42].) a = Carboxypenicillin; b = methylenampicillin; c = ampicillin; d = methicillin; e =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

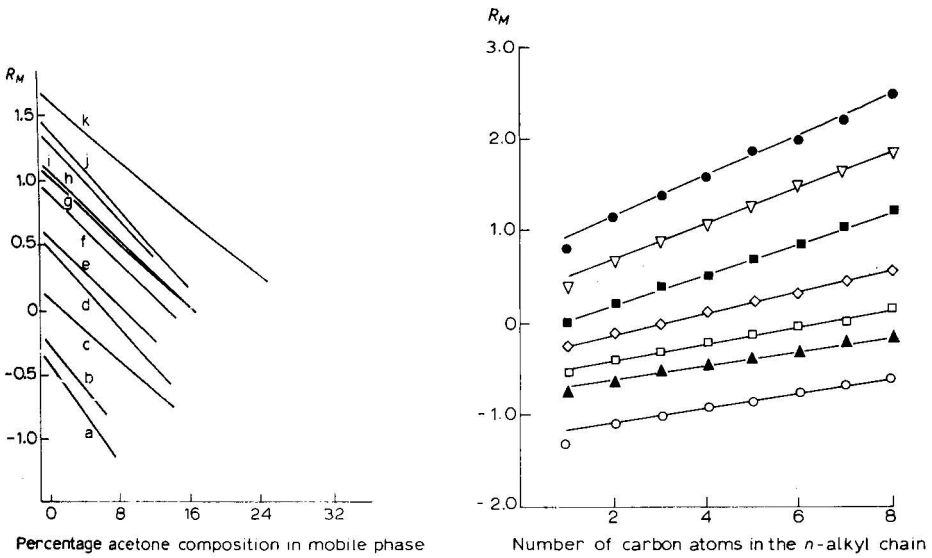


Fig. 3. Linear portions of the relationships between R_M values and percentage acetone composition derived from the data shown in Fig. 2. (After Biagi *et al.*[42].)

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 composition *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 $\Delta \log C_s$ 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 1

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	$R_{M(BAW)} = 1.604\ R_{M(diox)} - 0.606$	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

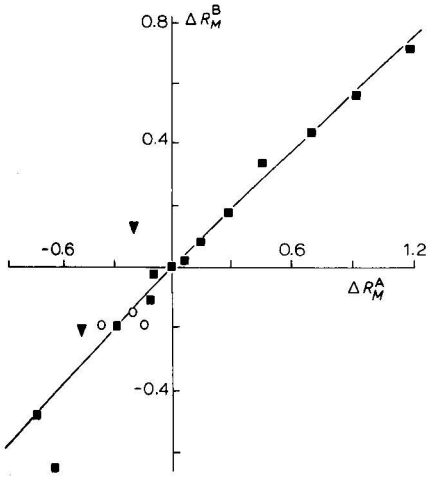


Fig. 5. Relationship between two sets of ΔR_M constants for a series of substituted acetanilides, as measured in a 1-octanol/acetone–water (1:9) system (A), and in a liquid paraffin/acetone–water (2:8) system (B). ■, *para* substituted compounds; ▼, *ortho* substituted compounds; ○, N-methyl-substituted 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. ΔR_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 —ring attached§	+0.305 (2) +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) Liquid paraffin/water–acetone (8:2) reversed phase, thin layer, 20° (ii) (K)	acetanilides —*para* attached	+0.143 (i) (2) +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 } no vicinal effects	**+0.165 (i) (2)**	**56**
		—(ii) 3-CH_3 group }	**+0.165 (ii) (2)**	
	System D	cresols, xylenols, phenols	+0.516§§ (i) (2)	
		—(i) *ortho*, (ii) *meta*, (iii) *para*	+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	−0.34§§ (2)	78
		—attached to a pyrimidine ring	−0.10§§ (2)	
		—(i) adjacent to a nitrogen atom		
		—(ii) *meta* to the nitrogen atoms		
	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)	77
			−0.20 (ii) (2)	
			−0.34 (iii) (2)	

(Continued on p. 22)

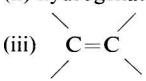
TABLE 2 *(continued)*

Functional group	*Chromatographic system**,**	*Solute and comments concerning the position of the functional group*	*ΔR_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)	acetanilides —*para* substituted	−0.114 (i) (2) −0.120 (ii) (2)	52
	System (N)	sulphonamides —(i) adjacent to a nitrogen atom in a pyrimidine ring —(ii) *meta* to the pyrimidine nitrogens	−0.370§§ (i) (2) −0.16 (ii) (2)	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)	acetanilides —*para*	−0.756 (i) (2) −0.502 (ii) (2)	52
	System M	phenols —(i) *para*, (ii) *meta*	−1.762 (i) (2) −1.720 (ii) (2)	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

	reversed phase, thin layer (Q)			
–N=CH₂ 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)**	**acetanilides** ***—para***	**+0.453 (i) (2)** **+0.342 (ii) (2)**	**52**
	System (O)	cinnamic acids	–0.46 (8)	79
	Polyamide gypsum/1-butanol–5 *N* NH_3 (100:33) (R)	*—ortho*-NO_2, adjacent to a hydroxyl group	+0.15[§§] (2)	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) System O	acetanilides —*para* β-aryl-*n*-butyric acids —*ortho*	+1.148 (2) +0.721 (2) +0.400§§ (2)	52 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) System F, with values at 100% water composition	acetanilides —*para* penicillins —introduced into an aliphatic chain	−0.672 (i) (2) −0.694 (ii) (2) −1.01 (2)	52 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.

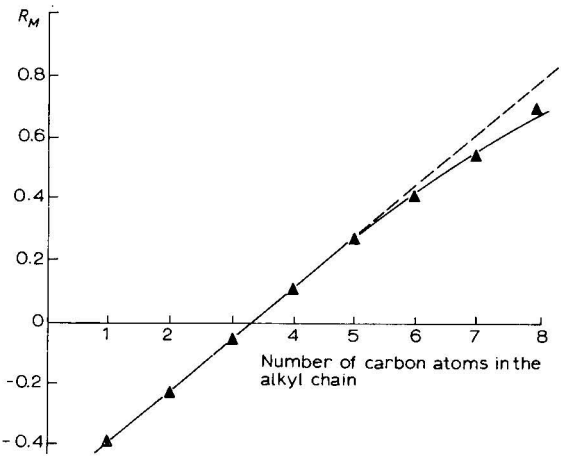


Fig. 6. Relationship between the number of carbon atoms in the alkyl chain of a series of 2-(1-substituted)-4,6-dinitrophenols and their R_M values as measured in a reversed-phase system. (After Clifford *et al.*[74].) Departure from linearity is thought to be due to a shielding effect by the alkyl chain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

(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

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

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

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

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:

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

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

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

where $C_{(\text{bio})}$ and $C_{(\text{water})}$ 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 $C_{(\text{bio})}$ and $C_{(\text{water})}$ 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

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

where $R_{M(\text{exp})}$ is the experimentally determined R_M value and $\log P_{(\text{bio})}$ 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

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

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 $\Delta R_{M(\text{1-octanol})}$ and $\pi_{(\text{1-octanol})}$. 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 $\pi_{(\text{1-octanol})}$ 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 $k/\Delta R_{M(\text{1-octanol})}$ 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.

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

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.*

$$\log BR = 0.416\ R_M + 0.295;\ n = 7;\ r = 0.934;\ s = 0.09 \tag{6.13}$$

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.*

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

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 $R_M + \sigma$ 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

$$PA = 28.42\,\Delta R_M^{A} + 26.47;\ n = 18;\ r = 0.986 \tag{6.22}$$

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

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

where ΔR_M^{A} 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].

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

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

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

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

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

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

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

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.

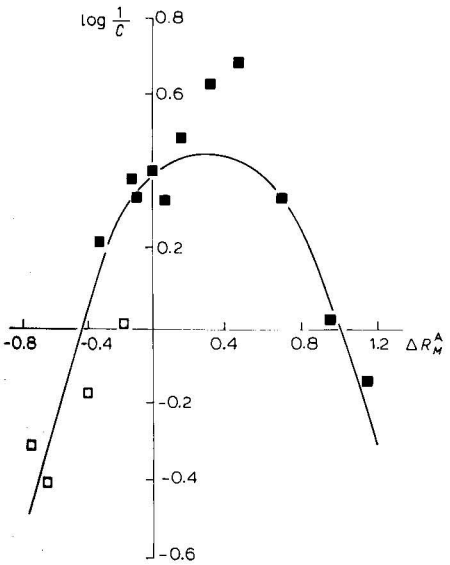


Fig. 7. Relationship between analgesic potency and the substituent constants ΔR_M^A for a series of *para* (■) and *ortho* (□) substituted acetanilides. The drawn curve has been generated from the regression equation found for the relationship (see text).

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) −0.052 (ii)	100
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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Journal of Chromatography, 113 (1975) 47–68
Chromatographic Reviews

CHREV. 83

STUDY OF CHARGE TRANSFER COMPLEXATION BY GAS–LIQUID CHROMATOGRAPHY

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(Received September 25th, 1974)

CONTENTS

1. INTRODUCTION

Gas–liquid chromatography (GLC) has been used for many years for the investigation of physico-chemical phenomena. In several cases, such studies have resulted in important advances in our understanding of solution phenomena. One such instance of current wide-spread interest to chemists of many disciplines is the GLC investigation of charge transfer complexation.

In a brief note, Benesi and Hildebrand[1] announced the presence of a newly discovered UV absorption band for a solution of iodine and benzene in 1948. There was evidence of a 1:1 complex between the two components, since the height of the band varied directly with the concentration of either component. One explanation of the phenomenon was to consider benzene as a Lewis base (electron donor), and iodine as a Lewis acid (electron acceptor); the resultant (charge transfer) complex can then be considered a Lewis acid–base adduct, even though only one electron (not an electron pair) is involved.

Since that time, hundreds of papers, many reviews, and at least five books[2–6]

have appeared which discuss charge transfer, and it is not surprising that gas chromatographers have also taken an interest in the subject. As early as 1958, Norman[7] reported the use of 2,4,7-trinitro-9-fluorenone (TNF) as a stationary phase for the separation of the three nitrotoluene isomers. Langer *et al.*[8] investigated di-*n*-alkyl tetrahalophthalates as selective phases for the separation of aromatic hydrocarbons in 1960; baseline resolution of *m*- and *p*-xylene was achieved with di-*n*-propyl tetrachlorophthalate in 90 min at 90°. Cooper and co-workers[9,10] later employed TNF for aromatic hydrocarbons and amines. Several workers have used inorganic salts as complexing agents, including Gil-Av and co-workers[11–15], van de Craats[16], Tenney[17], Bednas and Russell[18], Phillips[19], Muhs and Weiss[20], Banthorpe *et al.*[21], and Gump[22]. Kotsev and Shopov[23] have even studied olefin–liquid crystal complexation by GLC, where *p,p'*-azoxyphenetole in squalane was used as the stationary phase.

Since so many workers have investigated charge transfer complexation, it is somewhat surprising that there remains any disagreement about the nature of the interactions. Yet the authors[24–26] and others[27,28] currently claim that even today, 26 years after Benesi and Hildebrand's initial spectroscopic study of charge transfer behavior, this type of solution phenomenon is still not understood. Therefore, before we can review the study of complexation by GLC, we must first critically examine the nature of these interactions insofar as is possible, bearing in mind that currently accepted views may be substantially incorrect.

2. CHARGE TRANSFER COMPLEXATION: GENERAL CONCEPTS

Mulliken and Person[5] have presented the most recent summary of charge transfer considerations from a molecular orbital approach. If one molecule, D, donates an electron to a second molecule, A, the wave function of the complex, C, can be described as

$$\psi_N(\mathrm{C}) = a\psi_0(\mathrm{D,A}) + b\psi_1(\mathrm{D^+\text{–}A^-}) \tag{1}$$

where ψ_N is the total electronic ground-state wave function, ψ_0 is the (no-bond) wave function which describes all the intermolecular interactions except complexation, and ψ_1 is the (dative) wave function of complexation (as if complexation were the only force binding D and A together); a and b are weighting constants. The dative function, ψ_1, is written as a function of D^+ and A^- to indicate that transfer of charge from D to A causes appreciable ionization. If complete ionization does not occur (*i.e.*, if the complex is weakly held together), we represent eqn. 1 by:

$$\psi_N(\mathrm{C}) = a\psi_0(\mathrm{D,A}) + b\psi_1(\mathrm{D\text{–}A}) \tag{2}$$

Eqn. 2 will be used here, since only weak complexation will be considered.

As in any electronic description of molecular interactions, we can write the wave function of an excited state

$$\psi_V(\mathrm{C}) = -b^*\psi_0(\mathrm{D,A}) + a^*\psi_1(\mathrm{D^+\text{–}A^-}) \tag{3}$$

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where $\psi_V(C)$ is the excited-state electronic wave function, and $a^* \approx a \approx 1$ and $b^* \approx b \approx 0$. That is, when we promote (excite) an electron from D to A (by UV radiation for example) we cause appreciable ionization (charge transfer), and (D^+-A^-) is a more appropriate description of the complex than (D–A) or (D,A).

The energy of charge transfer, ΔE_{ct}, is just the difference between the energy levels of the electronic states

$$\Delta E_{ct} = E_V - E_N \tag{4}$$

and is readily found from the wavelength at which a complex absorbs light quanta

$$\Delta E_{ct} = h\nu_{ct} = \frac{hc}{\lambda_{ct}} \tag{5}$$

where ν_{ct} and λ_{ct} are the frequency and wavelength of charge transfer absorption, respectively. Note that ΔE_{ct} is not the energy initially required to form the complex, ΔE_f.

Rose[4] has reviewed the experimental observations of charge transfer phenomena: (1) The relation between charge transfer absorption frequencies and donor ionization potentials is generally (but not always) linear[29–31]. (2) The relation between charge transfer absorption frequencies and acceptor electron affinities is generally (but not always) linear[32]. (3) Donor ionization potentials and charge transfer equilibrium formation constants can sometimes be correlated[32–36]. (4) In weak complexes, dipole-induced dipole interactions account for most of the bonding (*i.e.*, $a \approx 1$, $b \approx 0$); for aromatic donor–acceptor systems, the dipole–induced dipole interactions are mainly electrostatic[37]. (5) There is generally no correlation between donor or acceptor dipole moments and charge transfer interactions[30]; there is a linear relation, however, between the dipole moment of the complex and the energy of charge transfer, and between the complex dipole moment and the donor ionization potential[37].

Several of these observations seem to be contradictory (for example, Nos. 4 and 5). To rationalize the apparent discrepancies, Mulliken and Person[5] have proposed the classification of donors and acceptors given in Table 1. Silver ion–olefin complexes are thus ν–$b\pi$ interactions, aromatic–aromatic complexes are $a\pi$–$b\pi$, and hydrogen bonding is classified as $a\sigma$–n. In the latter case, and in ν–n types (*e.g.*, $H_3N{:}BCl_3$), an electron pair may be involved, rather than just one electron. This breakdown of types helps to explain most of the above-noted experimental observations, since electrons are being removed from, and transferred into widely different

TABLE I

CLASSIFICATION OF DONORS AND ACCEPTORS[5]

Donors			*Acceptors*		
Electron taken from	*Type*	*Example*	*Electron goes to*	*Type*	*Example*
Non-bonding lone pair	n	$:NR_3$, RO:	Vacant orbital	ν	BCl_3, Ag^+
Bonding π orbital	$b\pi$	benzene, olefins	Anti-bonding σ orbital	$a\sigma$	I_2, R–H
			Anti-bonding π orbital	$a\pi$	TNF, fluoranil

types of molecular orbitals. However, some of the anomalies in the absorption spectra remain; for example, some types of complexes give two prominent charge transfer bands, while others give only one. To help explain these and other phenomena, Mulliken[5] proposed that there were fundamental (and usually sharply divided) degrees of charge transfer, which he called inner (strong, ionic), middle (transition), outer (weak, dative), and contact (random) complexes.

Inner (strong) complexes consist of two components which are largely ionized (D^+–A^-), whose spectra show bands for both the donor and acceptor ions (thus two bands per complex), and which may exhibit photoconduction, semiconduction, and paramagnetic properties (*e.g.*, tetramethyl-*p*-phenylenediamine–chloranil). Outer complexes are loosely held together by much weaker (dative) interactions, show the above properties of inner complexes to a much lesser extent (if at all), give only one prominent complex absorption band, and involve minimal transfer of charge in the electronic ground-state. Middle complexes lie between outer and inner complexes in the degree of charge transfer and are not generally distinguishable, since they are transitional electronic and geometrical configurations. Inner and outer complexes are strongly influenced by solvents; for example, tetramethyl-*p*-phenylenediamine–chloranil is an outer complex in cyclohexane, but forms inner complexes in more polar solvents[38], presumably because of ion stabilization by solvation. Finally, contact charge transfer results from random molecular collisions when both donor and acceptor species are present together in appreciable quantities; these interactions explain, for example, the "charge transfer" absorption bands of iodine–heptane and other pairs, which would not be expected to form complexes under normal conditions.

Thus, we can explain the above-noted experimental phenomena in terms of the type and relative strength of charge transfer interactions. For example, the donor and acceptor dipole moments are not related to the energy of charge transfer (*i.e.*, the frequency or wavelength at which the complex absorbs), because ΔE_{ct} depends only on the energy difference between the donor highest occupied molecular orbital and the acceptor lowest unoccupied molecular orbital, not on electrostatic attractive forces. Conversely, the dipole moment of the complex can be related to ΔE_{ct}, since it arises from an already partially transferred electron, and ΔE_{ct} is just the amount of energy needed to complete the process. We therefore find that the larger the complex dipole moment, the lower the energy of charge transfer[37].

The above classifications have not been accepted without criticism. Dewar and Thompson[39] found no correlation between tetracyanoethylene (TCNE)–aromatic hydrocarbon interaction strengths and absorption wavelengths, except that "... the points (with one doubtful exception) all lie in the same quadrant". Hassel and Rømming[40] proved via X-ray crystallography that the I–I axis lies perpendicular to the plane of the benzene ring in benzene–iodine complexes and not parallel to it, as Mulliken's treatment had earlier led him to postulate[33]. Nevertheless, the classification of donors and acceptors on the basis of molecular orbitals explains, for example, why Ag^+ forms complexes while alkali and alkaline earth ions do not. Including hydrogen bonding as merely a specific ($a\sigma$–n) type of charge transfer also allows us to explain the tendency of some donors and acceptors to form weak hydrogen bonds, while others [*e.g.*, pyridine–methyl iodide and $ROH{:}N(C_2H_5)_3$] form very strong ionic bonds[41,42]. The former are of course outer complexes, while the latter are inner complexes. Our rationale, then, for retaining the Mulliken theory of charge transfer is that

it fits most experimental observations, and those that it does not may be explained by our incomplete understanding of solution interactions.

3. SPECTROSCOPIC STUDIES

A. Ionization potentials, electron affinities, and formation constants

We now write the reaction between donor, D, and acceptor, A, to form complex, C, in the generalized form

$$D + A \rightleftharpoons C \tag{6}$$

for which the concentration equilibrium (formation) constant, K_f^c, is given by

$$K_f^c = \frac{[C]}{[D]\,[A]} = K_{eq.}\,\frac{\gamma_D^c \gamma_A^c}{\gamma_C^c} \tag{7}$$

where $K_{eq.}$ is the true thermodynamic equilibrium constant (defined in terms of activities, a_i), and γ_i^c is the concentration activity coefficient of the ith species. As noted earlier, the formation constant should depend at least in part on the ionization potential of the donor, and the electron affinity of the acceptor. [In the case of charge transfer, vertical[43–45] values should be used, since the electronic transitions occur approximately two orders of magnitude faster than nuclear transitions (the Franck–Condon principle). Vertical ionization potentials, I_v^d, and vertical electron affinities, E_v^a, are therefore employed throughout in this discussion; UV–photoelectron spectroscopy (PES) is now used to measure the former[46], while the latter can be inferred from charge transfer data[47].] However, attempts at correlating K_f^c, I_v^d, and E_v^a have generally proved fruitless. Bier[30] found no correlation between log K_f^x (the mole fraction formation constant) and ΔE_{ct} for *sym.*-trinitrobenzene (TNB)–aromatic hydrocarbons. Dewar and Thompson[39] found an approximately linear relation for log $[K_f^c/K_f^c$ (benzene)] *vs.* $[\lambda_{ct}-\lambda_{ct}$ (benzene)] for TCNE–methylbenzene complexes, but no such correlation was found when polycyclic aromatic hydrocarbon donors were used. Emslie *et al.*[48] found curved lines when log K_f^c was plotted *vs.* I_v^d for 26 alkylbenzene donors, and TNB and fluoranil acceptors. Several workers[32,49–57] have plotted the energy or frequency of charge transfer *vs.* the donor ionization potential with varying degrees of success. Plots of the charge transfer frequency[58] or the donor ionization potential[59] *vs.* the Gibbs free energy of formation, ΔG_f^0, however, have been shown to be linear for a variety of aromatic hydrocarbons. Some success has also been achieved with K_f^c (various acceptors) *vs.* K_f^c (TNB) plots[58,59].

In general, it can be said that ΔE_{ct}–K_f–I_v^d–E_v^a relations are tenuous at best, especially when K_f is determined via UV/visible or NMR spectroscopy. Some of the difficulties can undoubtedly be attributed to solvent effects, which are strong enough in some cases to stabilize outer → inner complexation transitions, as we noted earlier. We therefore now examine the solvent dependence of charge transfer behavior via the formation constant, K_f ,at the same time briefly presenting the spectroscopic techniques which have been (and are still being) employed to measure these values.

B. Solvent dependence of spectroscopic K_f values

All of the books[2–6] which have been written about charge transfer cite or fully develop the spectroscopic methods of measuring K_f values. Rose[4], in fact, lists more than twenty different methods which have been used. By far the most important are the UV/visible and NMR techniques, which are briefly summarized below.

(a) Benesi–Hildebrand[60] equation (UV/visible)

$$\frac{[A]_t b}{A_{ct}} = \frac{1}{\varepsilon_{ct} K_f^c [D]_t} + \frac{1}{\varepsilon_{ct}} \tag{8}$$

where b is the cell pathlength, $[A]_t$ and $[D]_t$ are the total amounts of acceptor and donor initially added to the solution, and A_{ct} and ε_{ct} are the complex absorbance and absorptivity, respectively. ($[D]_t$ is usually maintained in large excess over $[A]_t$ so that the approximation $[D]_t \approx [D]_{eq.}$ can be made). Eqn. 8 is in the form of $Y = mX + b$, so that when the left-hand side is plotted *vs.* $1/[D]_t$ ($[D]_t$ is varied while $[A]_t$ is held constant), a straight line of slope, $1/\varepsilon_{ct}K_f^c$, and intercept, $1/\varepsilon_{ct}$, is obtained.

(b) Scott[61] equation (UV/visible)

$$\frac{[A]_t[D]_t b}{A_{ct}} = \frac{1}{\varepsilon_{ct} K_f^c} + \frac{[D]_t}{\varepsilon_{ct}} \tag{9}$$

Eqn. 9 is obtained from eqn. 8 simply by multiplying the latter by $[D]_t$; it is an important modification, however, since the left-hand side is now plotted *vs.* $[D]_t$, and extrapolation is made to $[D]_t = 0$, not to $[D]_t = \infty$ ($1/[D]_t = 0$). The points at greater dilution are thus given more weight, where, presumably, Beer's law is more closely obeyed.

(c) Foster[62] equation (NMR)

$$\frac{1}{\Delta} = \frac{1}{\Delta_0 K_f^c [D]_t} + \frac{1}{\Delta_0} \tag{10}$$

$$\frac{\Delta}{[D]_t} = -K_f^c \Delta + K_f^c \Delta_0 \tag{11}$$

where Δ_0 is the difference between the chemical shift of pure acceptor and completely complexed acceptor ($\delta_A - \delta_C$), and Δ is the difference between the chemical shift of pure acceptor and acceptor at some value of $[D]_t$ ($\delta_{obs} - \delta_A$; $\delta_A > \delta_{obs} > \delta_C$). Eqns. 10 and 11 are the NMR analogues of eqns. 8 and 9; in the former, the left-hand side is plotted *vs.* $1/[D]_t$, and in the latter, *vs.* Δ.

The solvent dependence of formation constants determined by the above techniques is demonstrated in Table 2. There is an order of magnitude difference for many of the K_f values even with closely related solvents. The table also demonstrates that there is no correlation between UV/visible and NMR, regardless of the solvent used. Nor does it help to argue that mole fraction (K_f^x) or volume fraction (K_f^v) formation constants should be used[65,66] as Purnell and Srivastava have demonstrated[27];

TABLE 2

SOLVENT DEPENDENCE OF SPECTROSCOPIC FORMATION CONSTANTS

Donor	*Acceptor*	*Solvent*	*Temperature (°C)*	K_f	*Method*	*Reference*
Benzene	Iodine	CCl_4	22	1.72 l/mole	UV	60
		C_7H_{16}	22	1.15 l/mole	UV	60
Mesitylene	Iodine	CCl_4	22	7.2 l/mole	UV	60
		C_7H_{16}	22	5.3 l/mole	UV	60
N,N-Dimethylaniline	TNB	CCl_4	33.5	3.26 kg/mole	NMR	63
			33.5	2.04 l/mole	NMR	63
		$CHCl_3$	33.5	0.726 kg/mole	NMR	63
			33.5	0.455 l/mole	NMR	63
		CH_2Cl_2	33.5	0.399 kg/mole	NMR	63
			33.5	0.250 l/mole	NMR	63
Hexamethylbenzene	TNB	CCl_4	33.5	5.11 kg/mole	NMR	58
		CH_2ClCH_2Cl	33.5	0.59 kg/mole	NMR	58
Hexamethylbenzene	2,5-Dichloro-*p*-benzoquinone	CCl_4	33.5	1.92 kg/mole	NMR	58
		CH_2ClCH_2Cl	33.5	0.62 kg/mole	NMR	58
Hexamethylbenzene	1,4-Dinitrobenzene	CCl_4	33.5	1.01 kg/mole	NMR	58
		CH_2ClCH_2Cl	33.5	0.15 kg/mole	NMR	58
Hexamethylbenzene	Benzoquinone	CCl_4	33.5	0.66 kg/mole	NMR	58
		CH_2ClCH_2Cl	33.5	0.15 kg/mole	NMR	58
Phenanthrene	Pyromellitic dianhydride	$CHCl_3$	25.0	7.0 l/mole	UV	64
		CH_2Cl_2	25.0	2.6 l/mole	UV	64
		$(CH_3CO)_2O$	25.0	0.5 l/mole	UV	64
Durene	Pyromellitic dianhydride	CH_2Cl_2	25.0	1.3 l/mole	UV	64
		$(CH_3CO)_2O$	25.0	0.9 l/mole	UV	64
Naphthalene	Pyromellitic dianhydride	$CHCl_3$	25.0	2.8 l/mole	UV	64
		CH_2Cl_2	25.0	1.3 l/mole	UV	64
		$(CH_3CO)_2O$	25.0	0.7 l/mole	UV	64
Triphenylene	Pyromellitic dianhydride	$CHCl_3$	25.0	16.4 l/mole	UV	64
		CH_2Cl_2	25.0	4.4 l/mole	UV	64
		$(CH_3CO)_2O$	25.0	1.3 l/mole	UV	64
		C_6H_6	25.0	8.7 l/mole	UV	64
Fluoranthene	Pyromellitic dianhydride	$CHCl_3$	25.0	23.8 l/mole	UV	64
		CH_2Cl_2	25.0	7.9 l/mole	UV	64
		$(CH_3CO)_2O$	25.0	1.5 l/mole	UV	64
		C_6H_6	25.0	9.8 l/mole	UV	64
Fluorene	Pyromellitic dianhydride	$CHCl_3$	25.0	2.3 l/mole	UV	64
		CH_2Cl_2	25.0	1.4 l/mole	UV	64
		$(CH_3CO)_2O$	25.0	0.2 l/mole	UV	64

(Continued on p. 54)

TABLE 2 *(continued)*

Donor	*Acceptor*	*Solvent*	*Temperature (°C)*	K_f	*Method*	*Reference*
Hexamethylbenzene	Pyromellitic dianhydride	$CHCl_3$	25.0	2.2 l/mole	UV	64
		CH_2Cl_2	25.0	1.6 l/mole	UV	64
		$(CH_3CO)_2O$	25.0	1.3 l/mole	UV	64
Chrysene	Pyromellitic dianhydride	$CHCl_3$	25.0	23.3 l/mole	UV	64
		CH_2Cl_2	25.0	14.1 l/mole	UV	64
Benzo[*a*]anthracene	Pyromellitic dianhydride	$CHCl_3$	25.0	10.7 l/mole	UV	64
		CH_2Cl_2	25.0	6.2 l/mole	UV	64
		$(CH_3CO)_2O$	25.0	0.6 l/mole	UV	64
Pyrene	Pyromellitic dianhydride	$CHCl_3$	25.0	18.3 l/mole	UV	64
		CH_2Cl_2	25.0	9.0 l/mole	UV	64
		$(CH_3CO)_2O$	25.0	2.4 l/mole	UV	64
		C_6H_6	25.0	10.6 l/mole	UV	64
Anthracene	Pyromellitic dianhydride	$CHCl_3$	25.0	5.5 l/mole	UV	64
		CH_2Cl_2	25.0	3.7 l/mole	UV	64
		$(CH_3CO)_2O$	25.0	1.1 l/mole	UV	64
		C_6H_6	25.0	3.9 l/mole	UV	64
Perylene	Pyromellitic dianhydride	$CHCl_3$	25.0	57.8 l/mole	UV	64
		CH_2Cl_2	25.0	19.4 l/mole	UV	64
		C_6H_6	25.0	39.0 l/mole	UV	64
		CCl_4	33.5	15.4 kg/mole	NMR	59
Hexamethylbenzene	Fluoranil	$CHCl_3$	33.5	3.9 kg/mole	NMR	59
		CH_2ClCH_2Cl	33.5	3.6 kg/mole	NMR	59
		CH_2Cl_2	33.5	3.2 kg/mole	NMR	59
Pentamethylbenzene	Fluoranil	CCl_4	33.5	7.9 kg/mole	NMR	59
		$CHCl_3$	33.5	2.0 kg/ml	NMR	59
		CH_2ClCH_2Cl	33.5	1.6 kg/mole	NMR	59
		CH_2Cl_2	33.5	1.8 kg/mole	NMR	59
Durene	Fluoranil	CCl_4	33.5	4.9 kg/mole	NMR	59
		$CHCl_3$	33.5	1.3 kg/mole	NMR	59
		CH_2ClCH_2Cl	33.5	0.84 kg/mole	NMR	59
		CH_2Cl_2	33.5	0.85 kg/mole	NMR	59
Mesitylene	Fluoranil	CCl_4	33.5	2.2 kg/mole	NMR	59
		$CHCl_3$	33.5	0.68 kg/mole	NMR	59
p-Xylene	Fluoranil	CCl_4	33.5	1.5 kg/mole	NMR	59
		$CHCl_3$	33.5	0.42 kg/mole	NMR	59
Toluene	Fluoranil	CCl_4	33.5	0.96 kg/mole	NMR	59
		$CHCl_3$	33.5	0.25 kg/mole	NMR	59
Benzene	Fluoranil	CCl_4	33.5	0.70 kg/mole	NMR	59
		$CHCl_3$	33.5	0.16 kg/mole	NMR	59
Hexamethylbenzene	1,4-Dicyano-2,3,5,6-tetrafluorobenzene	CCl_4	33.5	5.2 kg/mole	NMR	59
		$CHCl_3$	33.5	0.92 kg/mole	NMR	59
		CH_2ClCH_2Cl	33.5	0.72 kg/mole	NMR	59
		CH_2Cl_2	33.5	0.71 kg/mole	NMR	59

TABLE 2 *(continued)*

Donor	*Acceptor*	*Solvent*	*Temperature (°C)*	K_f	*Method*	*Reference*
Pentamethylbenzene	1,4-Dicyano-2,3,5,6-tetrafluorobenzene	CCl_4	33.5	3.4 kg/mole	NMR	59
		$CHCl_3$	33.5	0.64 kg/mole	NMR	59
		CH_2ClCH_2Cl	33.5	0.44 kg/mole	NMR	59
		CH_2Cl_2	33.5	0.48 kg/mole	NMR	59
Durene	1,4-Dicyano-2,3,5,6-tetrafluorobenzene	CCl_4	33.5	2.4 kg/mole	NMR	59
		$CHCl_3$	33.5	0.46 kg/mole	NMR	59
		CH_2ClCH_2Cl	33.5	0.26 kg/mole	NMR	59
		CH_2Cl_2	33.5	0.35 kg/mole	NMR	59
Mesitylene	1,4-Dicyano-2,3,5,6-tetrafluorobenzene	CCl_4	33.5	1.5 kg/mole	NMR	59
		$CHCl_3$	33.5	0.29 kg/mole	NMR	59
p-Xylene	1,4-Dicyano-2,3,5,6-tetrafluorobenzene	CCl_4	33.5	1.2 kg/mole	NMR	59
		$CHCl_3$	33.5	0.26 kg/mole	NMR	59
Hexamethylbenzene	TNB	CCl_4	33.5	5.1 kg/mole	NMR	59
		$CHCl_3$	33.5	0.86 kg/mole	NMR	59
		CH_2ClCH_2Cl	33.5	0.59 kg/mole	NMR	59
Pentamethylbenzene	TNB	CCl_4	33.5	3.1 kg/mole	NMR	59
		$CHCl_3$	33.5	0.67 kg/mole	NMR	59
		CH_2ClCH_2Cl	33.5	0.43 kg/mole	NMR	59
Durene	TNB	CCl_4	33.5	2.1 kg/mole	NMR	59
		$CHCl_3$	33.5	0.49 kg/mole	NMR	59
		CH_2ClCH_2Cl	33.5	0.33 kg/mole	NMR	59

TABLE 3

FORMATION CONSTANTS FOR NAMED AROMATIC HYDROCARBONS WITH TNF AT 40° (REF. 27)

Solvent	*Donor*	K_f *(l/mole)*		K_f		K_f	
		UV	*NMR*	*UV*	*NMR*	*UV*	*NMR*
Di-*n*-butyl succinate	Toluene	0.116	−0.019	−0.045	−0.624	1.087	−0.168
	m-Xylene	0.210	0.072	0.210	0.072	1.670	0.565
	o-Xylene	0.167	0.105	0.241	−0.033	1.357	0.850
Di-*n*-butyl adipate	Toluene	−0.030	−0.010	−0.710	−0.571	−0.272	−0.087
	m-Xylene	0.082	0.096	−0.246	−0.198	0.654	0.769
	o-Xylene	—	—	—	—	—	—
Di-*n*-butyl sebacate	Toluene	−0.008	0.053	−0.730	−0.519	−0.075	0.491
	m-Xylene	0.065	0.041	−0.448	−0.448	0.522	0.333
	o-Xylene	0.145	0.098	−0.180	−0.356	1.177	0.800

their UV and NMR data for K_f^x and K_f^v are shown in Table 3. Many of the values are negative, which is physically meaningless.

Clearly, these are somewhat distressing results, particularly since most of the theory about charge transfer is based on spectroscopic data. The validity of comparisons between other methods and spectroscopic values is also open to serious question. For example, Bertrand and co-workers[67] recently reported the determination of the pyridine/iodine formation constants in cyclohexane and carbon tetrachloride; their results, along with cited spectroscopic values, are shown in Table 4. Although the agreement is good, it may only be fortuitous, given the data in Tables 2 and 3.

TABLE 4

COMPARISON OF CALORIMETRIC K_f^c VALUES FOR PYRIDINE–IODINE WITH SPECTROSCOPIC DATA AT 25°

	K_f^c(l/mole)	
	Calorimetry	*Spectroscopy*
Cyclohexane	124[67]	135[68]
Carbon tetrachloride	103[67]	102[68]
	108[70]	101[69]

4. GAS CHROMATOGRAPHIC STUDIES

A. Classification of experimental methods

Purnell[71] has presented a classification of donor–acceptor–solvent interactions with which the various GLC techniques may be distinguished. These have recently been reviewed by Wellington[72], and so are only briefly considered here; for donor (D) solutes and acceptor (A) stationary phase (S) additives:

Class A. Solute reacts with stationary phase additive to give complexes of the type D_mA_n, where $m, n \geqslant 1$.

Class B. Solute reacts with stationary phase to give complexes of the type S_pD_m, where $m, p \geqslant 1$.

Class C. Solute polymerizes or depolymerizes in solution.

Class D. Additive reacts with stationary phase to give complexes of the type S_pA_n.

Wellington[72] has added:

Class E. Solvated donor, D_mS_x, reacts with solvated additive, A_nS_y, to form solvated complexes, $C_{m:n}S_z$, giving up qS solvent molecules in the process.

(a) Class A: Method of Gil-Av and Herling[13]

For 1:1 Class A interactions, solute (donor) solubility in the stationary phase is enhanced by the presence of a complexing (acceptor) additive, so that the distribution coefficient becomes:

$$K_L = \frac{\text{solute concentration in the stationary phase}}{\text{solute concentration in the gas phase}} = \frac{[D]_L^0 + [C]}{[D]_M} \qquad (12)$$

where $[D]_L^0$ is the equilibrium amount of free donor in solution, and $[D]_M$ is the total donor concentration in the gas phase. Multiplying by $[D]_L^0[A]/[D]_L^0[A]$ gives:

$$K_L = \frac{[D]_L^0}{[D]_M} + \frac{[D]_L^0}{[D]_M} \frac{[C]}{[D]_L^0[A]} [A] = K_L^0 + K_L^0 K_f^c [A] \tag{13}$$

where K_L^0 is the solute distribution coefficient in the absence of additive, [A]. ([A] must be present in excess over [C] to ensure that $[A] \approx [A]_{eq.}$).

Eqn. 13 was first presented by Gil-Av and Herling[13] in 1962, and yields formation constants from the slope/intercept quotient of K_L *vs.* [A] plots. The equation was originally employed to study Ag^+–olefin complexation (ethylene glycol stationary phase), but has since been used by many workers for various organic acceptor additives and donor solutes. (Note that the additive need not be the acceptor; the choice of which complex component to dissolve in the liquid phase is in fact purely a matter of convenience, and for donor additives, [A] is replaced by [D] in eqn. 13.) Wellington[72] has summarized the GLC data that have been obtained via eqn. 13, and Purnell[71] and Wellington[72] have commented on its applications and limitations.

(b) Class B: method of Martire and Riedl[73]

There is seemingly no way to get at formation constants when pure complexing agent is used as the stationary phase (Class B). However, Martire and Riedl[73] showed that:

$$K_{eq.} = \left(\frac{1}{\bar{A}\,\gamma_A}\right)\left(\frac{V_g^A\,V_g^C}{V_g^B\,V_g^D} - 1\right) \tag{14}$$

where $K_{eq.}$ is the true thermodynamic equilibrium constant, γ_A and $\bar{A}$ are the activity coefficient and molar volume of the pure (acceptor) complexing phase, V_g^A and V_g^B are the specific retention volumes of an inert (non-complexing) solute on inert and complexing phases, respectively, and V_g^C and V_g^D are the specific retention volumes of a complexing solute on the same stationary phases. The (Raoult's law) activity coefficient, γ_A, is given by[73]

$$\gamma_A = \frac{V_g^B\,MW_C}{V_g^A\,MW_N} \tag{15}$$

where MW_C and MW_N are the molecular weights of the complexing and inert stationary phases, respectively. If the (donor) solute and complex are at infinite dilution, $\gamma_{D,C}^{\infty} \to 1$ (Henry's law), $K_{eq.}$ is related to K_f^c by

$$K_{eq.} = \frac{[C]}{[D]\,a_A} = K_f^{c\prime}/\bar{A}\,\gamma_A = K_f^c/\gamma_A \tag{16}$$

where a_A is the activity of the neat (acceptor) stationary phase. Liao *et al.*[74] have shown that eqn. 16 is valid when the inert reference phase is identical in all respects to the complexing phase, except that the latter forms complexes while the former does not. While this is a rather stringent requirement of the reference phase, the method

has been used with excellent success to measure charge transfer interactions[74–78], and promises to become a very important technique for the determination of $K_{eq.}$ values. Indeed, eqn. 14 is the only method developed to date by which $K_{eq.}$ can be found.

5. COMPARISON OF METHODS

A. GLC and spectroscopy

The only comparison of GLC and spectroscopic data thus far is that by Purnell and Srivastava[27]. Their GLC concentration formation constant (K_f^c) data for the same solvents and compounds as in Table 3 are now given in Table 5. The values are all positive, but the most remarkable feature of these data is that, even for the same compounds, solvents, and temperature, results by the same workers in the same laboratory suggest that UV and NMR data are not valid. The GLC results, on the other hand, are all positive, decrease with increasing temperature[27], and appear to be physically meaningful.

TABLE 5

GLC[27] FORMATION CONSTANTS FOR NAMED COMPOUNDS WITH TNF AT 40°

Solvent	*Donor*	K_f^c *(l/mole)*
Di-*n*-butyl succinate	Benzene	0.590
	Toluene	0.702
	m-Xylene	0.825
	o-Xylene	0.871
	p-Xylene	0.764
	Ethylbenzene	0.615
Di-*n*-butyl adipate	Benzene	0.481
	Toluene	0.491
	m-Xylene	0.615
	o-Xylene	0.606
	p-Xylene	0.624
	Ethylbenzene	0.448
Di-*n*-butyl sebacate	Benzene	0.353
	Toluene	0.332
	m-Xylene	0.401
	o-Xylene	0.393
	p-Xylene	0.425
	Ethylbenzene	0.355

B. GLC: Class A and Class B

According to eqn. 16, when $\gamma_A = 1$, the equilibrium constant should be identical to the concentration formation constant. That is, the Gil-Av–Herling method (Class A) should give the same results (for the same solutes and complexing solvents) as the Martire–Riedl method (Class B). The only test of this hypothesis (given by eqn. 16) is by Liao *et al.*[74], who used di-*n*-octylmethylamine as the complexing phase, *n*-octadecane as the inert or reference phase, and $CHCl_3$, CH_2Cl_2, and CH_2Br_2 as

the complexing solutes. γ_D was found to be 0.993 ± 0.002 via eqn. 15, in which case $K_{eq.}$ values should be identical to K_f^c values. Their results are given in Table 6, where the agreement is seen to be excellent, indicating that the two GLC methods offer consistent results, further strengthening our contention that GLC data are a valid measure of charge transfer interactions.

TABLE 6

COMPARISON[74] OF $K_{eq.}$ AND K_f^c WHEN $\gamma_D = 1$

Solute	K_f^c *(l/mole) (eqn. 13)*	$K_{eq.}$ *(eqn. 14)*
$CHCl_3$	0.405 ± 0.019	0.403 ± 0.006
CH_2Cl_2	0.179 ± 0.014	0.187 ± 0.004
CH_2Br_2	0.222 ± 0.004	0.219 ± 0.004

6. RATIONALIZATION OF THE DIFFERENCE BETWEEN GLC AND SPECTROSCOPIC DATA: SOLVATION

We now explore possible explanations for the discrepancy between GLC and spectroscopic values. In both UV and NMR studies, donors, acceptors, and complexes exist in solution as solvated species such that interactions must occur through shells of solvent molecules surrounding each component. For strong (inner) complexes, some solvent molecules may be removed so that donor and acceptor are in direct contact; for weak (outer) complexes, this may or may not be true. We assume, for now, that for 1:1 complexes, the following reactions occur in solution:

$$A + S_n \leftrightharpoons AS_n \tag{17}$$

$$D + S_m \leftrightharpoons DS_m \tag{18}$$

$$AS_n + DS_m \leftrightharpoons CS_p + qS \tag{19}$$

Thus, formation of a complex is an interaction between solvated A and D which gives solvated C plus q solvent molecules which have been cast off (or added, in which case q is negative) such that: $n + m = p + q$. Carter *et al.*[79] and others[80–82] have pointed out that solvent effects must be considered whenever weak interactions are measured spectroscopically, but few workers have taken notice of this fact. Yet the work of Carter *et al.*[79] offers a very straightforward method of determining the extent of solvation, as well as solvent-independent formation constants. We therefore now examine the technique of Carter *et al.* in an attempt to explain the differences between spectroscopic and GLC data.

The formation constant, K_f^c, is now defined in terms of eqn. 19

$$K_f^c = \frac{[CS_p]\,(X_s)^q}{[AS_n]\,[DS_m]} \tag{20}$$

where X_s is the free solvent mole fraction, given by

$$X_s \simeq \frac{[S]_{free}}{[S]_t + [D]_t + [A]_t} \tag{21}$$

$[S]_{free}$ is the concentration of free solvent at equilibrium, and $[S]_t$ is the total solvent concentration. X_s rather than [S] is used in eqn. 20 so that the formation constant will retain units of 1/mole, and can therefore be compared to the Benesi–Hildebrand equation. When $[D]_t \gg [A]_t$, $[D]_t = [D]_{eq.}$, and

$$\frac{[A_t]b}{A_{ct}} = \frac{(X_s)^q}{\varepsilon_{ct}K_f^c[D]_t} + \frac{1}{\varepsilon_{ct}} \tag{23}$$

The only difference between eqn. 23 and the original Benesi–Hildebrand relation, eqn. 8, is the appearance of $(X_s)^q$ in the numerator of the first term on the right-hand side. Eqn. 8 failed to include solvent effects, which is a serious omission: if we assume that eqns. 17–19 are reasonable (*i.e.*, if a compound dissolves in a solvent it becomes solvated by that solvent), then according to eqn. 19 as more donor is added to a solution containing an acceptor, complex CS_p is formed and qS amount of solvent is released, thus diluting what we had assumed was a constant $[A]_t$. The freshly added donor also takes up some amount of solvent to form DS_m, further compounding the problem. Let us represent $[S]_0$ as the free solvent concentration when $[D]_t = 0$ but after $[A]_t$ has been added to the solution. Assuming that the change in the total solution volume is negligible when $[D]_t$ is added

$$[S]_{free} = [S]_0 - [D]_t\left(\frac{\overline{V}_D}{\overline{V}_S}\right) \tag{24}$$

where $\overline{V}_D$ and $\overline{V}_S$ are the donor and solvent molar volumes, whose ratio we conveniently represent by λ

$$[S]_{free} = [S]_0 - \lambda[D]_t \tag{25}$$

Eqn. 25 merely says that the total amount of solvent in the solution remains constant

$$\overline{V}_S[S]_{free} + \overline{V}_D[D]_t = \overline{V}_S[S]_0 \tag{26}$$

(Note that $[S]_0 > [S]_{free}$.) $[S]_{free}$ is now given by

$$\begin{aligned}[S]_{free} &= [S]_t - n[A]_t - m[D]_t + q[C] \\ &= [S]_0 - \lambda[D]_t - m[D]_t - n[A]_t + q[C] \\ &= [S]_0 - (m + \lambda)[D]_t - n[A]_t + q[C]\end{aligned} \tag{27}$$

Substituting eqn. 27 into eqn. 21 yields

$$\begin{aligned}X_s &= \frac{[S]_0 - (m + \lambda)[D]_t - n[A]_t + q[C]}{[S]_t + [D]_t + [A]_t} \\ &= \frac{[S]_0 - (m + \lambda)[D]_t}{[S]_0 + (1 - \lambda)[D]_t}\end{aligned} \tag{28}$$

since $n[A]_t \ll [D]_t < [S]_0 \approx [S]_t$. Eqn. 23 now becomes

$$\frac{[A]_t b}{A_{ct}} = \frac{1}{\varepsilon_{ct}K_f^c[D]_t}\left[\frac{1 - (m + \lambda)[D]_t/[S]_0}{1 + (1 - \lambda)[D]_t/[S]_0}\right]^q + \frac{1}{\varepsilon_{ct}} \tag{29}$$

Since $[S]_0 \gg [D]_t$, and neglecting higher terms

$$\frac{[A]_t b}{A_{ct}} = \frac{1}{\varepsilon_{ct} K_f^c [D]_t} [1 - q(m + \lambda)([D]_t/[S]_0)][1 - q(1 - \lambda)([D]_t/[S]_0)] + \frac{1}{\varepsilon_{ct}}$$

$$= \frac{1}{\varepsilon_{ct} K_f^c [D]_t} + \frac{1}{\varepsilon_{ct}} \left[1 - \frac{q(m + 1)}{K_f^c [S]_0}\right] \quad (30)$$

The formation constants of eqns. 8 and 30 are related by

$$K_f^c \text{ (eqn. 8)} = K_f^c \text{ (eqn. 30)} - \frac{q(m + 1)}{[S]_0} \quad (31)$$

and

$$\varepsilon_{ct} \text{ (eqn. 8)} = \varepsilon_{ct} \text{ (eqn. 30)} \frac{K_f^c \text{ (eqn. 30)}}{K_f^c \text{ (eqn. 8)}} \quad (32)$$

K_f^c and ε_{ct} (eqn. 8) are thus underestimated and overestimated, respectively, and the Benesi–Hildebrand equation will only be approximately correct when

$$K_f^c \gg q(m + 1)/[S]_0 \quad (33)$$

i.e., when complexation is strong. For the cases of weak or contact charge transfer

$$K_f^c < \frac{q(m + 1)}{[S]_0} \quad (34)$$

may be true, and the Benesi–Hildebrand equation will fail badly.

Carter *et al.*[79] tested the validity of eqn. 30 by plotting ε_{ct} *vs.* K_f^c for methylbenzenes–iodine, TNB, and chloranil, each in CCl_4 solvent, for which $[S]_0$ is given by density/molecular weight = 10.3 moles/l. (Recall that $[S]_0 > [D]_t \gg [A]_t$.) If Beer's law is correct, and if K_f^c is measured at a wavelength at which only charge transfer interactions cause absorption, then

$$\text{as } K_f^c \to 0, \ \varepsilon_{ct} \to 0 \quad (35)$$

Carter *et al.* found that this was obeyed for each set of methylbenzenes/acceptor data only at discrete values, namely, $q(m + 1) = 9$ (iodine), 30 (TNB), and 6 (chloranil). The largest change is for TNB, and we therefore assume that it is the most solvated, while chloranil is the least solvated. Further evidence of the validity of eqn. 30 was found when the gas-phase data of Lang and Strong[83] for benzene–iodine were compared to the liquid-phase data in CCl_4. Assuming $q(m + 1) = 9$, $\varepsilon_{ct}^{\text{liquid}}$ was found to be 2400, whereas $\varepsilon_{ct}^{\text{gas}}$ was 1700; $\varepsilon_{ct}^{eqn.\ 8}$ was 17,000 when solvation effects were not considered.

Clearly, solvent effects are responsible for most of the anomalies in Table 2, but may be removed by the treatment of Carter *et al.*; it is remarkable, in fact, that many more investigations have not been in this direction. Assuming discrete solvation shells surrounding the donor, acceptor, and complex moieties, one can also rationalize differences between UV and NMR data. In the former, electronic transitions form inner complexes which may have different geometrical configurations (and

most certainly have different electronic configurations) than the ground state. The accuracy of UV K_f^c values therefore depends implicitly on how closely related the solvated electronic ground state is to the solvated electronic excited state. In the NMR technique, chemical shifts depend on solvent shielding effects, which can be appreciably different even for closely related solvents[6]. Thus, the UV and NMR techniques are at variance simply because solvent effects are manifested differently in each; that is, even the same solvent will affect electronic transitions differently than it will chemical shifts, because two fundamentally different properties are being measured.

We now consider GLC data. Eqn. 13 allows the determination of all solution effects except the change in X_s; as in eqn. 23, varying [A] will alter $[S]_{free}$, so that K_L^0 will not be a true constant. Meen[84] and Wellington[72] are thus far the only workers who have considered the application of the argument of Carter *et al.* to GLC. For an acceptor additive and donor solutes

$$K_L = K_L^0 \left[1 + \frac{K_f^c [AS_n]}{(X_s)^q}\right]$$

$$= K_L^0 \left\{1 + K_f^c [A]_t \left[1 - \frac{[A]_t q(n+1)}{[S]_t}\right]\right\} \tag{36}$$

where $(X_s)^q$ is approximately given by

$$(X_s)^q \approx 1 + \left[\frac{[A]_t\, q(n+1)}{[S]_t}\right] \tag{37}$$

analogous to eqns. 21 and 28. Note that the term $q(n + 1)$ and not $q(m + 1)$ is used here, since in GLC the acceptor is in large excess over the donor, not *vice versa* as in spectroscopy. If $[A]_t \ll [S]_t$, eqn. 36 reduces to eqn. 13, the Gil-Av–Herling relation, which will usually be the case if less than 0.2 *M* solutions of A in S are employed. "Best" values of $q(n + 1)$ should be available from spectroscopic data via the method of Carter *et al.*[79], so that eqns. 30 and 36 should now yield identical K_f^c values, regardless of the solvent or method. Purnell[85] has very recently applied these considerations to NMR equations as well, and does indeed find that GLC and spectroscopic data are identical when solvent effects are taken into account. This is the most exciting development yet in the study of charge transfer complexation, and will clearly be applied much more so in the future than in the past; workers in the field will finally have a means whereby formation constant data from many different techniques can be compared on a common basis, and we anticipate great strides in solution theory in the very near future as a result.

7. DETERMINATION OF PHYSICO-CHEMICAL PROPERTIES VIA GLC COMPLEXATION STUDIES

A. Vertical ionization potentials and electron affinities

If true charge transfer forces are operative, we would expect the formation

constant to be a function of the donor vertical ionization potential, as we noted earlier

$$K_f^c = F(I_v^d) \tag{38}$$

To establish that this is the case, we have examined several types of donors on different complexing phases[24–26]. The results are encouraging: the lower the ionization potential, the larger the formation constant. Data by Meen *et al.*[86] also indicate that K_f^c is a function of I_v^d. This variation has in fact been used in a very recent publication[25] to determine vertical ionization potentials: the GLC K_f^c data of butadienes with known[87–92] I_v^d values were plotted as K_f^c *vs.* I_v^d at three temperatures. The lines were curved, and so a non-linear least-squares treatment[93,94] was necessary to fit the data. The approximate equation constants were:

$$45°: K_f^c = -9.075 \times 10^{-3} (I_v^d)^2 + 0.750 \tag{39}$$

$$50°: K_f^c = -9.237 \times 10^{-3} (I_v^d)^2 + 0.750 \tag{40}$$

$$55°: K_f^c = -9.445 \times 10^{-3} (I_v^d)^2 + 0.750 \tag{41}$$

where TNF in di-*n*-butyl phthalate (DNBP) was used as the stationary phase. To ensure that eqns. 39–41 were good approximations, the known ionization potentials were back-calculated from the respective formation constants at each temperature; the known and averaged values agreed to 1.02% at worst, and generally much better than that. To ascertain the accuracy of the GLC-determined I_v^d values, we have collaborated with Heilbronner and Bieri[95] in obtaining PES data for the dienes whose ionization potentials were previously unknown; the results are presented in Table 7, where the difference between the GLC and PES values for each compound, δ, is also given. The first four compounds agree to within $\pm$ 0.10 eV, a remarkable feat since the GLC instrument we used was by no means a precision device, and many of the formation constants bordered on the experimental error of K_f^c (determined to be

TABLE 7

COMPARISON OF PES AND GLC I_v^d VALUES[25,95]

Diene	I_v^d *(eV)*		
	PES	*GLC*	δ *(eV)*
cis-1,3-Pentadiene	8.61 (*trans*)	8.65	0.04
2-Ethyl-1,3-butadiene	8.79	8.76	0.03
2-Methyl-1,3-pentadiene	–	8.53	–
3-Methyl-1,3-pentadiene	8.40	8.51	0.11
4-Methyl-1,3-pentadiene	8.45	8.49	0.04
1,3-Hexadiene	8.53	8.70	0.17
1,3-Heptadiene	8.51	8.75	0.24
1-Methoxy-1,3-butadiene	8.26	7.98	0.28
5-Methyl-1,3-hexadiene	8.47	8.81	0.34
2,4-Dimethyl-1,3-pentadiene	9.31	8.85	0.46
2,4-Heptadiene	8.14	8.71	0.57

$\pm$ 0.010 l/mole). The remaining compounds disagree by increasing amounts, the worst case being 2,4-heptadiene. We have attributed these δ values to steric hindrance to charge transfer, and will discuss them shortly. Meanwhile, where no anomalous (*e.g.*, steric) effects occur, GLC can be used to determine vertical ionization potentials to $\pm$ 0.1 eV (PES data are usually accurate to $\pm$ 0.02–0.03 eV).

Charge transfer forces should also be proportional to the acceptor electron affinity

$$K_f^c = F(E_v^a) \tag{42}$$

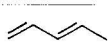
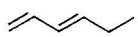
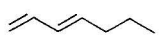
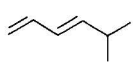
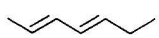
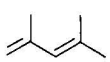
No study has appeared which uses eqn. 42, but our K_f^c data[25] for aromatic hydrocarbons and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in DNBP are two to three times as large as those by Meen *et al.*[86], even if normalized to the same K_L^0 values. DDQ has an electron affinity of 1.95 eV, compared to 1.00 eV for TNF[47], and so the results are entirely as expected. The relation could perhaps be improved if solvation were taken into account (recall that TNB and chloranil differed by $\Delta[q(m+1)] = 24$ solvation molecules); we are now examining closely related classes of acceptors, for example, the pyridazinediones, for which no E_v^a data exist[96,97], but which could in principle be found via GLC, analogous to our procedure for I_v^d values.

B. Substituent effects and steric hindrance to charge transfer

The values of Table 7 are now presented in a different manner, in Table 8. For the first four compounds, as the substituent on the end of the butadiene skeleton be-

TABLE 8

EFFECTS OF STERIC HINDRANCE ON CHARGE TRANSFER

Donor	δ *(eV)*
	0.04
	0.17
	0.24
	0.34
	0.57
	0.46
	0.03

comes larger, the difference between PES and GLC I_v^d values increases. Since K_f^c is proportional to $(I_v^d)^{-1}$, we note that if steric hindrance (or other factors) causes a decrease in K_f^c, I_v^d will be increased by a similar amount, resulting in δ values larger than 0.1 eV (the experimental error of the GLC method). The δ values then become a measure of steric hindrance to charge transfer. Bulky end-groups clearly appear to hinder (planar) complex formation with TNF, but a large δ value is also found for 2,4-dimethyl-1,3-pentadiene. This compound is known to be twisted about the central single bond[98] and is thus partially deconjugated, which results in a higher ionization potential than expected. Conversely, 2-ethyl-1,3-butadiene does not appear to be sterically hindered, which is most surprising. Although further work is needed to verify this result, we are forced to postulate that charge transfer in butadiene–TNF complexes is an end–on interaction, rather than planar–planar, as has historically been assumed. We are therefore now investigating 2-alkyl-1,3-pentadienes to confirm this finding.

In another recent paper[26], we attempted to measure out-of-plane deformation angles for a series of β-ionones via the Class B technique of Martire and Riedl. Aromatic hydrocarbons were first examined with di-*n*-butyl tetrachlorophthalate, and $K_f^{c\prime}$ (eqn. 16) was shown to vary inversely as I_v^d, as expected. Next, a series of substituted aromatic amines was investigated, and out-of-plane substituents at the nitrogen[99] were shown to profoundly affect charge transfer behavior. Finally, out-of-plane twisting for the β-ionone series was measured by NMR[100,101], but could not be correlated to GLC $K_f^{c\prime}$ values because the angles were too severe ($\approx 30°$). An upper limit of the GLC method was thus established to be approximately 10°–15°. Work is now under way with the compounds described by Forbes *et al.*[98] to further clarify the usefulness of GLC for the determination of out-of-plane deformation angles.

8. FUTURE AREAS OF INVESTIGATION

Several approaches to the question of charge transfer now become apparent. The method of Martire and Riedl[73] offers great promise for the evaluation of thermodynamic equilibrium constants. A modified Gil-Av and Herling equation which includes solvent effects (eqn. 30) also appears to be an extremely useful approach which will enable results from different experimental methods to be compared. Eon and Guiochon[28] and Martire[102] have very recently presented a theoretical treatment of this problem, and Purnell *et al.*[85] have been able to show that GLC and spectroscopic data do indeed yield identical results when solvation effects (determined via the method of Carter *et al.*[79]) are taken into account. Liao and Martire[77] have begun to investigate (hydrogen bonding) complexation in the light of acid–base theory[103], and we[25,26] have shown that many molecular properties can also be deduced from GLC charge transfer data, including ionization potentials, electron affinities, steric factors, out-of-plane deformations, and so forth.

Finally, several new approaches await investigation. The question of end–on *vs.* planar intermolecular interaction looms as a most important study, since the very nature of charge transfer may thereby be elucidated. Another study that would be most interesting is the illumination of a glass capillary GLC column during the elution of complex-forming donors. Suppose, for example, that the liquid phase was DDQ

in DNBP, and benzene, toluene, and the three xylenes were being chromatographed. $\lambda_{max.}$ for aromatic hydrocarbons–DDQ differs by over 200 nm in some cases[56,104], being 427 nm for benzene and 450 nm for toluene ($CHCl_3$ solvent[56]). Suppose that we now irradiate the glass GC column at 420 nm, well away from the toluene and xylene maxima, but close enough to benzene to produce an outer → inner complex transition. Benzene should then be strongly retained, while the other solutes will elute unaffected by the illumination. If this does not occur, then charge transfer theory as we know it is incorrect, and the entire subject would require complete re-evaluation. If benzene is strongly retarded, the difference, ΔK_L, between "dark" and "illuminated" distribution coefficients should be a good measure of the strength (hence ε_{ct}) of charge transfer interactions, which could easily be verified by UV studies. Conversely, it may be possible to obtain ε_{ct} values at infinite dilution via GLC, which can only be done indirectly (by extrapolation to [D] = 0; Beer's law) in UV. Illumination may also be used as an added dimension for difficult separations. Meen *et al.*[86] have evaluated the use of complexing agents in analytical GC applications, and we[25] have shown that even DDQ in high concentrations will not be of much use in adding to column selectivity. The ability to cause inner complex transitions by UV/visible irradiation, however, may considerably brighten the outlook on this approach. Lastly, while we have limited the discussion here to GLC, there is every reason to expect that high-performance liquid–liquid chromatography will prove equally as useful[105]. Gil-Av *et al.*[106] have already begun complexation studies by high-performance liquid–liquid chromatography, and it has been suggested[107] that solvation effects could greatly improve separations when complex-forming stationary phases are used in this technique. In short, the study of charge transfer is currently in a high state of flux, and offers every promise of being one of the most rewarding physicochemical topics yet investigated by gas (and liquid) chromatographers.

9. SUMMARY

The study of charge transfer complexation by gas–liquid chromatography (GLC) is presented. The GLC results differ significantly from spectroscopic data, and it is argued that the chromatographic technique seems to be valid, whereas other methods are at best questionable. Very recent data by the authors also indicate that much more information is available from GLC studies than had previously been recognized, such as the determination of vertical ionization potentials, vertical electron affinities, molecular substituent and out-of-plane deformation effects, and steric hindrance to charge transfer.

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Journal of Chromatography, 113 (1975) 69–95
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CHREV. 84

USE OF SE-30 AS A STATIONARY PHASE FOR THE GAS–LIQUID CHROMATOGRAPHY OF DRUGS

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(Received November 11th, 1974)

CONTENTS

1. INTRODUCTION

Gas–liquid chromatography (GLC) has for many years been chosen by numerous analysts as the method of choice for screening extracts of biological materials for the presence of drugs. The technique is reliable, highly selective and very sensitive, allowing qualitative analyses to be performed on submicrogram amounts of drugs. A large number of stationary phases have been advocated for use and over 300 different phases have been reported[1]. This makes the analyst's task very difficult when faced with a large amount of retention data in the literature — some in retention times, some in relative retention times to a number of different drugs and some in retention indices, all on numerous stationary phases. What is required is the standardisation not only on one or two stationary phases, but also on the retention parameters to be measured. When this is achieved, a comprehensive collection of retention data can be compiled which can be continuously and easily updated.

Fortunately, a large step forward has already been taken by the suggestion that a list of "preferred stationary phases" be designated and that all work should be carried out using them[1–4]. A previous study in this laboratory[5] compared eight different stationary phases and found that a low polarity phase, such as SE-30 or OV-17, is the "preferred liquid phase" for the identification of basic drugs. Of all the low-polarity phases in common use SE-30 is undoubtably the best choice since it was one of the first phases to be used[6], is probably the most extensively used for the analysis of drugs and is the stationary phase for which most data have been reported[7–11]. Chemically, SE-30 is a dimethyl silicone elastomer, with a useful temperature range of 80–300° and is often sold as the proprietary E-301 or OV-1 gum rubbers, the liquid silicone OV-101 being very similar.

The monographs concerning the GLC analysis of drugs[12,13], as well as the published collections of drug retention data using SE-30 (refs. 14–16), quote data as

retention times or relative retention times to a number of drugs. In the author's opinion retention indices provide the most reproducible GLC retention parameters for drug identification and this study has therefore been carried out using retention indices. The intention of this work was to convert literature retention data using SE-30 as the stationary phase for drugs and other commonly occurring chemicals such as plasticisers into retention indices and to compile these as a list for use in identification procedures for drugs. Naturally, variations between laboratories will occur with measurements of retention indices due to differences in chromatographic conditions such as temperature, sample size and column loading and the inter-laboratory variations of measurements of retention indices for drugs under normal operational conditions have been determined.

2. EXPERIMENTAL

Three drugs (amphetamine, diphenhydramine and dipipanone) were sent as aqueous solutions (1 mg/ml) to eleven laboratories. Each laboratory was asked to extract the drugs, chromatograph them on the normal SE-30 column used in their laboratory (Table 1) and report the results as retention indices for the three drugs.

Published data were used to accumulate a library of retention indices for drugs on SE-30 columns. Where collections of data were in retention times or relative retention times, the retention indices were calculated after calibration curves of retention times plotted against known retention indices had been constructed. For those commonly used drugs not included in the literature, or where two literature values for a drug differed by more than 50 retention index units, the retention indices were measured in this study using the conditions for laboratory 2 (Table 1).

3. RESULTS AND DISCUSSION

The individual retention indices obtained by each laboratory for each of the

TABLE 1

SE-30 GLC SYSTEMS USED

Mean temperatures used: amphetamine, 100°; diphenhydramine, 200°; dipipanone, 230°.

Laboratory	*Column*						
	Material	*Length (m)*	*I.D. (mm)*	*Phase*	*Support*	*Carrier*	*Flow-rate (ml/min)*
1	—	—	—	OV-1	—	—	—
2	Glass	2	4	2% SE-30	Chromosorb G	N_2	60
3	Glass	2	3	2.5% SE-30	Chromosorb G	N_2	44
4	Glass	2	4	2.5% SE-30	Chromosorb W	N_2	40
5	Glass	1.5	2	2.5% SE-30	Chromosorb G	N_2	60
6	Glass	2	4	2% SE-30	Chromosorb G	N_2	60
7	Glass	1.5	4	2.5% E-301	Chromosorb G	Ar	75
8	Glass	1.5	3	2.5% SE-30	Celite	N_2	60
9	Glass	1.5	4	2.5% SE-30	Diatomite CQ	N_2	50 and 25
10	Glass	2	3	3% E-301	Chromosorb W	N_2	18
11	Glass	1.5	4	2.5% SE-30	HP Chromosorb W	N_2	40

TABLE 2

SE-30 RETENTION INDICES

Laboratory	*Amphetamine*	*Diphenhydramine*	*Dipipanone*
1	1110	1855	—
2	—	—	2467
3	1135	1880	2500
4	1170	1870	2480
5	1165	1895	2515
6	1148	1864	2496
7	1140	1870	2492
8	1168	1897	2492
9	1153	1897	2477
10	1170	1892	2502
11	1140	1850	2507
Mean	1150	1877	2492
Standard deviation	19.4	17.8	14.6
Coefficient of variation	1.7	0.95	0.58

drugs on the SE-30 columns, together with the respective means, standard deviations and coefficients of variation are given in Table 2. All the columns were made of glass and contained packing material of 2–3% SE-30 (Table 1). The support appeared to make little difference to the results, although there was a tendency to use Chromosorb G, acid washed, dimethyldichlorosilane treated, 80–100 mesh. The standard deviation decreased as the retention index increased, which supports earlier findings that SE-30 acts more satisfactorily and reproducibly at higher temperatures[5]. Each analyst can form his own decision as to the likely deviation of his results on his

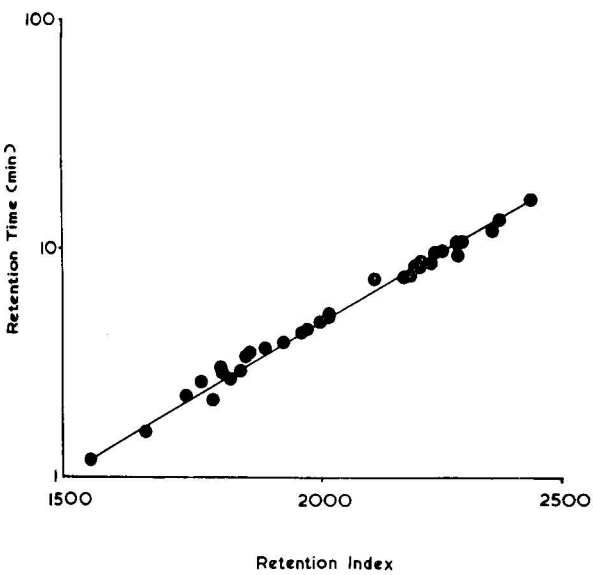

Fig. 1. Calibration graph of experimentally determined retention indices for a number of drugs against the retention time data of Beckett and Moffat[8] using SE-30 as the stationary phase.

TABLE 3

RETENTION INDICES OF 480 COMPOUNDS USING SE-30 AS THE STATIONARY PHASE, ARRANGED IN ALPHABETICAL ORDER OF DRUG NAME

Neg. = No peak between 1000 and 3500.

Drug	*Retention index*	*Reference*	
		Source	*Check*
Acenocoumarin	1900	10	
Acepromazine	2665	8, 10, 15	
Acetanilide	1365	10	
Acetophenazine	Neg.	This study	10
Acetyldihydrocodeinone	2450	15	
Acetylsalicylic acid	1295	10, 19	
Aconitine	2280	19	
Adiphenine	2190	19	10
Aldrin	1950	19	
Aletamine	1280	7, 10	
Allobarbitone	1600	10	15
Allylbarbituric acid	1670	10	15
Alphacetylmethadol	2160	15	
Alphameprodine	1840	15	
Alphamethadol	2150	15	
Alphaprodine	1895	10, 15	
Ametazole	1390	This study	
Amethocaine	2215	19	10, 15
Amidopyrine	1890	19	8, 10
p-Aminosalicylic acid	1330	10	
Amisometradine	2025	10	
Amitryptyline	2200	19	8, 10, 15
Amolanone	2210	10, 15	
Amphetamine	1110	19	7, 10
Amprotropine	2010	15	
Ampyrone	1950	19	
iso-Amylamine, *see* Isoamylamine			
Amylobarbitone	1725	19	10, 15
Amylocaine	1635	8, 10	
Anileridine	2845	19	10, 15
Aniline	1150	10	
Anisaldehyde	1230	10	
Anisinidione	2285	10	
Antazoline	2330	19	8, 10, 15
Anthracene	1790	10	
Apoatropine	2025	15	
Aprobarbitone	1620	10	15
Atropine	2175	19	10, 15
Azacyclonol	2210	19	10, 15
Azapetine	1925	15	
Barbitone	1495	19	10, 15
Bemegride	1390	10	
Benactyzine	2230	19	10
Benzethidine	2680	15	
Benzhexol	2230	19	10, 15
Benzocaine	1530	This study	8, 10
Benzphetamine	1850	7, 10	

TABLE 3 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Benztropine	2320	10, 15	
Betameprodine	1830	15	
Betaprodine	1790	15	
Biperiden	2280	15	
Bromodiphenhydramine	2150	19	10, 15
Bromopheniramine	2070	10, 15	
Brucine	3280	19	
Buclizine	3285	19	10
Bufotenine	2000	15	
Buphenine	2320	This study	15
	1270*	This study	
Bupivacaine	2270	8	15
Butabarbitone	1655	10	
Butacaine	2470	5	10
Butallylonal	2025	10	
Butanilicaine	2010	15	
Butethamate	1740	15	
Butethamine	2050	This study	10, 15
Butobarbitone	1660	10	15
Butriptyline	2155	15	
Butylphthalate	1880	10	
n-Butyric acid	1330	10	
Caffeine	1810	19	8, 10, 15
Camphor	1130	10	
Cantharidin	1490	19	
Capric acid	1485	10	
n-Caproic acid	1410	10	
Captodiame	2775	19	10, 15
Caramiphen	1950	15	
Carbamazepine	2290	15	
Carbaryl	1490	19	
Carbetapentane	2240	This study	10
Carbimazole	1670	15	
Carbinoxamine	2050	5	10, 15
Carbromal	1500	19	
Carisoprodol	1850	19	10
Carminic acid	1690	10	
Carphenazine	3590	19	
Chlophedianol	2070	15	
Chlorbenside	2050	19	
Chlorcyclizine	2215	19	8, 10, 15
Chlordane	2020	19	
Chlordiazepoxide	2790	This study	19
	2500*	This study	10, 15
	2460*	This study	
	2300*	This study	15
Chlormethiazole	1230	20	
Chlormezanone	2250	10	
Chloroprocaine	2200	10, 15	

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TABLE 3 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Chloropyrilene	2130	This study	10
Chloroquine	2575	19	8, 15
Chlorothymol	1510	10	
Chlorphenesin carbamate	1690	10	
Chlorpheniramine	2000	19	8, 10, 15
Chlorphenoxamine	2040	10, 15	
Chlorphentermine	1320	19	7, 10, 15
Chlorpromazine	2440	19	8, 10, 15
Chlorpropamide	1740	10	15
Chlorprothixene	2510	10	
Chlorzoxazone	1710	10, 15	
Cholesterol	3015	10	
Cinchocaine	2690	19	10, 15
Cinchonidine	2625	19	8, 15
Cinchonine	2575	19	8, 10, 15
Clemizole	2680	5	10, 15
Clofibrate	1560	10	
Cocaine	2180	19	8, 10
Codeine	2385	19	8, 10, 15
Cotarnine	1780	19	
Cotinine	1670	8	
Cyclandelate	1900	10	
Cyclizine	2010	19	8, 10, 15
Cyclobarbitone	1950	10	15
Cyclomethycaine	2225	10	
Cyclopentamine	1080	This study	7, 10
Cyclopentolate	2010	15	
Cypenamine	1345	7	
Cyproheptadine	2355	10, 15	
Dapsone	2860	19	10
Deptropine	2590	15	
Desipramine	2260	This study	10, 15
Desmethylchlorpromazine	2480	8	
Desomorphine	2275	15	
Dexamethasone	2950	10	
Dexoxadrol	2340	8	
Dextromethorphan	2115	8, 15	
Dextropropoxyphene	2180	19	8, 10, 15
Diamorphine	2615	19	8, 10, 15
Diazepam	2410	5	10, 15
Diazinon	1760	10	
Dibenzepin	2480	10	
Dicophane	2290	19	10, 15
Dicylomine	2100	This study	10
Didesmethylchlorpromazine	2480	8	
Dieldrin	2110	19	15
Diethazine	2280	15	
Diethylpropion	1480	19	7, 10
Diethylthiambutene	2000	15	
Diethyltryptamine	1900	10	
Dihydrocodeine	2365	8	10, 15

TABLE 3 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Dihydrocodeinone	2425	10	
Dihydroergotamine	2310	19	
Dihydromorphine	2440	15	
Dimenhydrinate	1840	10	
Dimethindene	2270	10, 15, 19	
Dimethoxanate	2030	This study	
3,4-Dimethoxyphenethylamine	1540	19	
Dimethrin	1210	19	
N,N-Dimethylamphetamine	1230	7	
N,N-Dimethylphenethylamine	1150	10	
Dimethylthiambutene	1870	15	
Dimethyltryptamine	1750	10, 15	
4,6-Dinitro-*o*-cresol	1620	10	
2,4-Dinitrophenol	1510	10	
Diphenadione	2910	10	
Diphenhydramine	1855	19	8, 10
Diphenylpyraline	2090	This study	10, 15
Dipipanone	2470	5	8, 15
Dopamine	2150	15	
Doxapram	2875	15	
Doxylamine	1925	10, 15	
Dyclonine	1640	10	
Ectylurea	1360	19	10
Embramine	2150	15	
Emcyclamate	1090	10	
Ephedrine	1350	19	7, 10, 15
Ergocristine	2500	10	
Ergocryptine	2180	10	
Ergotamine	2360	10	
Etafedrine	1460	7, 15	
Ethchlorvynol	1030	19	
Ethinamate	1360	19	10
Ethinyloestradiol	2710	10	
Ethoheptazine	1845	19	8, 10, 15
Ethomoxane	1975	15	
Ethopropazine	2350	This study	10, 15
Ethotoin	1800	10	
Ethoxyquin	2800	19	
Ethoxzolamide	2550	10	
N-Ethylamphetamine	1210	19	7, 10
N-Ethylbenzylamine	1010	10	
Ethylisobutrazine	2455	8	
Ethylmorphine	2415	19	10, 15
Ethylmethylthiambutene	1925	15	
Etoxeridine	2310	15	
Etryptamine	1850	8	
Eucatropine	2000	15	
Fenfluramine	1220	7, 10	
Fenmetramide	1765	8	

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TABLE 3 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Fentanyl	2700	15	
Fluopromazine	2175	15	
Fluphenazine	3045	19	
Gallamine	2700	15	
Gentisic acid	2350	10	
Glutethimide	1825	19	8, 10, 15
Guanethidine	Neg.	This study	
Haloperidol	2940	10	
Harmine	2280	This study	10
Heptabarbitone	2100	10	
Hexachlorophane	2795	10, 15	
Hexamine	1210	10	
Hexobarbitone	1850	10	
Hexoestrol	2400	10	
Hexylcaine	1950	15	
Hippuric acid	1730	10	
Histamine	1500	10	
Homatropine	2045	19	10, 15
Hydrallazine	1530	10	
Hydrastine	2975	19	10
Hydrastinine	1590	19	
Hydrocodone	2425	15	
Hydromorphone	2490	10	
Hydroxyamylobarbitone	1930	15	
Hydroxychloroquine	2860	19	10
Hydroxypethidine	2025	15	
Hydroxyphenamate	1740	19	10, 15
p-Hydroxyphenylpyruvic acid	1380	10	
Hydroxyquinidine	2780	15	
Hydroxyzine	2840	19	10, 15
Hyoscine	2285	19	10, 15
Hyoscyamine	2225	19	10
Imipramine	2220	19	8, 10, 15
Indomethacin	2690	10	
Iproniazid	1580	This study	10
Isoamylamine	1025	10	
Isocarboxazid	1960	19	10
Isomethadone	2125	8	
Isomethepten	1050	7, 10	
Isoniazid	1630	19	10
Isoprenaline	1720	10	
Isopropamide	2060	10	
Isopropylhexedrine	1140	7	
Isoquinoline	1440	10	
Isothipendyl	2260	This study	8, 10, 15
Ketamine	1830	10, 15	
Ketobemidone	2010	15	
Lachesine	1860	15	
Lauric acid	1600	10	

TABLE 3 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Leptazol	1535	This study	7, 10
Levallorphan	2340	5	10, 15
Levomethorphan	2230	15	
Levorphanol	2225	10, 15	
Lignocaine	1860	19	8, 10, 15
Lindane	1740	10	
cis-Linoleic acid	1330	10	
Linolenic acid	2175	10	
Lobeline	1780	19	
Lysergide	3445	19	
Malathion	1900	10	
Mandelic acid	1500	10	
MDA (Methylenedioxyamphetamine)	1470	19	10
Mebhydrolin	2450	15	
Mebutamate	1865	19	10
Meclofenoxate	1740	15	
Meclozine	3050	19	10, 15
Mefenamic acid	2185	10, 15	
Mephenesin	1550	10	
Mephenesin carbamate	1570	10	
Mephenoxalone	2120	19	10
Mephentermine	1240	19	7, 10
Mepivacaine	2075	This study	10, 15
Meprobamate	1790	19	8, 10, 15
Mepyramine	2205	5	8, 10, 15
Mescaline	1690	19	10, 15
Metabutoxycaine	2225	10	
Metaxalone	2180	10	
Methadone	2170	19	8, 10, 15
Methadone (cyclic metabolite)	2030	8	
Methaphenilene	1980	10	
Methapyrilene	1970	19	8, 15
Methaqualone	2180	10, 15	8
Metharbitone	1470	10	15
Methdilazine	2470	8, 10, 15	
Methimazole	1550	15	
Methixene	2490	10	
Methocarbamol	1510	10	
Methohexitone	1760	10	
Methoin	1795	19	10
Methotrimeprazine	2515	This study	8, 10, 15
Methoxamine	1720	10, 15	
Methoxychlor	2410	19	10
Methoxyphenamine	1360	7, 10	
Methoxypromazine	2500	19	10
Methsuximide	1595	19	
Methylaminomethylheptane	1000	7	
Methylamphetamine	1170	19	7, 10
Methyldesorphine	2290	15	

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TABLE 3 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Methyldihydromorphine	2375	15	
Methyldimethoxyamphetamine, *see* STP			
Methylenedioxyamphetamine, *see* MDA			
Methylephedrine	1400	7, 10	
Methylphenidate	1780	19	7, 10
Methylphenobarbitone	1920	19	15
Methylsalicylate	1200	10	
Methyltestosterone	2610	15	
Methyprylone	1505	19	10, 15
Methysergide	3050	10	
Metopon	2375	15	
Metronidazole	1590	10, 15	
Modaline	1420	7	
6-Monoacetylmorphine	2480	15	
Morpheridine	2500	15	
Morphine	2435	19	8, 10, 15
Myristic acid	1740	10	
Nalorphine	2570	19	10, 15
Naphazoline	2090	This study	10
Nialamide	1500*	This study	
Nicotinamide	1460	10	
Nicotine	1340	19	7, 10
Nicotinyl alcohol	1150	10, this study	
Nicoumalone	1770	10	
Nifenazone	1600	15	
Nikethamide	1500	19	7, 10
Nitrazepam	2675	5	15
p-Nitromethylamphetamine	1655	10	
Norethynodrel	2520	10	
Norfenfluramine	1130	7	
Normethadone	2080	8	
Norpethidine	1745	19	8
Norpseudoephedrine	1310	7	
Nortriptyline	2215	5	10, 15
Noscapine	3100	10	
Nystatin	1960	10	
Orphenadrine	1925	5	8, 10, 15
Oxazepam	2335	10	15
Oxycodone	2425	15	
Oxymetazoline	2170	10, 15	
Oxymorphone	2520	10, 15	
Oxypertine	2125	15	
Oxyphencyclimine	2540	10	
Pachycarpine	1765	19	
Palmitic acid	1975	10	
Papaverine	2805	5	10, 15
Paracetamol	1710	19	10
Parathion	1925	10	
Pargyline	1200	19	7, 10

TABLE 3 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Pecazine	2550	19	10, 15
Pentachlorophenol	1740	This study	10
Pentaquin	2540	15	
Pentazocine	2265	19	8, 10, 15
Pentobarbitone	1750	19	10, 15
Perphenazine	2200	19	
Pethidine	1740	19	8, 10, 15
Phenacaine	2615	10	
Phenacetin	1660	19	8, 10, 15
Phenadoxone	2510	8, 15	
Phenanthrene	1780	10	
Phenazocine	2670	8, 10	
Phenazone	1830	19	10
Phenazopyridine	2345	10	
Phencyclidine	1870	19	10, 15
Phendimetrazine	1440	19	7, 10, 15
Phenelzine	1340	This study	10
α-Phenethylamine	1010	19	10
β-Phenethylamine	1120	19	10
Pheneturide	1450	15	
Phenindamine	2160	This study	8, 10, 15
Pheniprazine	1410	10	
Pheniramine	1805	5	8, 15
Phenmetrazine	1430	19	7, 10
Phenobarbitone	1950	19	10, 15
Phenothiazine	2010	10, 15	
Phenoxybenzamine	2230	10, 15	
Phenoxypropazine	1465	7	
Phenprobamate	1520	15	
Phensuximide	1640	10	
Phentermine	1130	19	7, 10
Phenylbutazone	2370	10, 15	
Phenylpropanolamine	1310	19	7, 10
Phenylsalicylate	1740	10	
Phenyltoloxamine	1925	10, 15	
Phenyramidol	2000	This study	10
Phenytoin	2335	15, 19	
Pholcodine	2380	10	
Physostigmine	1810	19	10
Picrotoxin	2205	19	
Pilocarpine	2010	10	15
Pipamazine	3260	This study	
Pipazethate	2010	15	
Piperidolate	2325	This study	10, 15
Piperocaine	1955	This study	10
Piperoxan	1830	15	
Pipethanate	2470	10	
Pipobroman	2200	10	
Pipradrol	2150	19	8, 10, 15

(Continued on p. 80)

TABLE 3 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Pramoxine	2305	This study	10, 15
Prenylamine	2540	15	
Prilocaine	1845	8, 10	
Primaquine	2320	This study	10
Primidone	2250	19	8, 10, 15
Probarbital	1550	19	10, 15
Probenecid	2320	This study	10
Procainamide	2230	10, 15	
Procaine	1995	19	8, 10, 15
Procarbazine	1990	10	
Prochlorperazine	2935	10	
Procyclidine	2115	This study	10, 15
Progesterone	2785	10	
Promazine	2295	19	8, 10, 15
Promethazine	2260	19	8, 10, 15
Propantheline	2350	10	
Properidine	1740	15	
Propiomazine	2725	This study	15
Propoxycaine	2320	15	
Propranolol	2145	10, 15	
N-Propylamphetamine	1330	19	
Propylhexedrine	1170	19	7, 10
5-Propyl-5-isobutylbarbituric acid	1690	10	
Prothipendyl	2330	This study	10, 15
Protokylol	1500	10	
Protriptyline	2230	15	
Pseudoephedrine	1350	7	
Pyrathiazine	2520	8, 10	
Pyridoxamine	2000	19	
Pyrimethamine	2140	19	10
Pyrrobutamine	2430	19	8, 10, 15
Quinalbarbitone	1775	19	10, 15
Quinidine	2760	19	10, 15
Quinine	2755	19	10, 15
Quinoline	1440	10	
iso-Quinoline, see Isoquinoline			
Quinuronium	2100	15	
Resorcinol	1610	10	
Rotenone	3250	10	
Salicylamide	1460	19	10
Salicylic acid	1330	19	10
Sanguinarine	2880	19	
Santonin	2160	10, 15	
Secbutobarbitone	1780	19	
Sparteine	1770	10, 15	
Stearic acid	2175	10	
Stilboestrol	2300	This study	10, 15
STP (Methyldimethoxyamphetamine)	1620	19	10
Strychnine	3040	19	10, 15

TABLE 3 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Styramate	1670	10	
Sulphonal	1475	15	
Tacrine	2140	15	
Talbutal	1700	10	15
Tetrahydrocannabinol	2455	19	
Tetrahydrozoline	1960	8	
Thebacon	2500	15	
Thebaine	2525	19	10
Thenyldiamine	2010	This study	10
Theobromine	1840	10	
Theophylline	1485	10	
Thiabendazole	2010	10	
Thiamylal	1890	19	10
Thiantoin	2145	19	
Thiethylperazine	3260	10	
Thiopentone	1850	19	10
Thiopropazate	3450	This study	
Thioridazine	3110	19	10
Thiosalicylic acid	1500	10	
Thiothixene	3015	10	
Thonzylamine	2200	This study	10, 15
Thymol	1270	10	
Thymoxamine	1830	15	
Tolazamide	1650	10	
Tolazoline	1550	15	
Tolbutamide	1690	10	
Tranylcypromine	1210	19	7, 10
Triamcinolone	3070	10	
Trifluoperazine	2680	This study	8, 10, 15
Triflupromazine	2210	19	8, 10
Trimeperidine	1830	15	
Trimeprazine	2320	This study	8, 10, 15
Trimethoprim	2610	15	
Trimipramine	2200	15	
Tripelennamine	1960	19	8, 10, 15
Triprolidine	2250	19	8, 10, 15
Tropine	1200	10	
Troxidone	1100	19	
Tryptamine	1740	8, 10, 15, 19	
Tybamate	1700	10	
Tymazoline	1850	8	
Vinbarbitone	1750	10	15
Warfarin	1460	19	10, 15
Xenysalate	2440	15	
Xylometazoline	1920	10, 15	

(Continued on p. 82)

TABLE 3 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Yohimbine	3290	19	10
Zoxazolamine	1625	10	

* Major decomposition product.

column from those in Tables 3 and 4 by consideration of the standard deviations in Table 2 and this is discussed later.

Retention indices for 171 drugs were available from previously published data (Moffat *et al.*[5] and Kazyak and Permisohn[9]) and the retention indices for a further 43 drugs were measured for this study. The data for these 214 drugs were used to calibrate other published data (Beckett *et al.*[7], Beckett and Moffat[8], Finkle *et al.*[10] and Clarke[15]) to convert their retention times and relative retention times to retention indices. Fig. 1 shows such a calibration graph and all values were within $\pm$ 50 retention index units from the regression line. Tables 3 and 4 give the retention indices of 480 drugs and chemicals using SE-30 as the stationary phase arranged in alphabetical order of drug name and ascending order of retention index, respectively. No attempt has been made to separate particular chemical groups, so that the tables contain acidic, neutral and basic drugs as well as steroids, insecticides, plasticisers and other commonly encountered chemicals. They are named according to Martindale[17], or the Merck Index[18] if they are not included in the former's work. Some entries are for the parent drugs although the peaks actually observed were thermal decomposition products. These are indicated where known, but care must be exercised when using a linked GC–MS system because the peak observed may not be for the parent drug. For example, quinuronium is a quaternary ammonium compound and would not be expected to be eluted from a GC column although it gives a peak of retention index 2100.

The source of each retention index is included in the tables. In some cases the mean values obtained from several sources have been used. Often a check on the value finally included was made from another source and the results had to agree to within $\pm$ 50 retention index units. Where values obtained from different sources varied by more than $\pm$ 50 retention index units, the value finally used was determined experimentally. Only 9 out of 480 values needed to be re-determined.

Table 3 can be used to find the retention index of a drug, or if the drug is not in the table its probable retention index can be obtained by using the correlation graph of molecular weight *vs.* retention index (Fig. 2). Compounds with many polar functional groups will deviate to the largest extent from the regression line.

The reproducibility of retention indices will change with the temperature of the column (Table 2) and also with the nature of the drug being chromatographed. Those drugs containing alkyl, aryl, alkoxy, amide or tertiary amino groups will give nearly symmetrical peaks and the retention indices will be very reproducible. On the other hand, primary amino, alcoholic and especially phenolic groups will cause tailing peaks in the chromatogram. In these cases, and also where tailing peaks are obtained

TABLE 4

RETENTION INDICES OF 480 COMPOUNDS USING SE-30 AS THE STATIONARY PHASE, ARRANGED IN ASCENDING ORDER OF RETENTION INDEX

Neg. = No peak between 1000 and 3500.

Drug	*Retention index*	*Reference*	
		Source	*Check*
Acetophenazine	Neg.	This study	10
Guanethidine	Neg.	This study	
Methylaminomethylheptane	1000	7	
N-Ethylbenzylamine	1010	10	
α-Phenethylamine	1010	19	10
Isoamylamine	1025	10	
Ethchlorvynol	1030	19	
Isomethepten	1050	7, 10	
Cyclopentamine	1080	This study	7, 10
Emcylamate	1090	10	
Troxidone	1100	19	
Amphetamine	1110	19	7, 10
β-Phenethylamine	1120	19	10
Camphor	1130	10	
Norfenfluramine	1130	7	
Phentermine	1130	19	7, 10
Isopropylhexedrine	1140	7	
Aniline	1150	10	
N,N-Dimethylphenethylamine	1150	10	
Nicotinyl alcohol	1150	10, this study	
Methylamphetamine	1170	19	7, 10
Propylhexedrine	1170	19	7, 10
Methylsalicylate	1200	10	
Pargyline	1200	19	7, 10
Tropine	1200	10	
Dimethrin	1210	19	
N-Ethylamphetamine	1210	19	7, 10
Hexamine	1210	10	
Tranylcypromine	1210	19	7, 10
Fenfluramine	1220	7	10
Anisaldehyde	1230	10	
Chlormethiazole	1230	20	
N,N-Dimethylamphetamine	1230	7	
Mephentermine	1240	19	7, 10
Thymol	1270	10	
Buphenine	1270*	This study	
Aletamine	1280	7, 10	
Acetylsalicylic acid	1295	10, 19	
Norpseudoephedrine	1310	7	
Phenylpropanolamine	1310	19	7, 10
Chlorphentermine	1320	19	7, 10, 15
p-Aminosalicylic acid	1330	10	
n-Butyric acid	1330	10	
cis-Linoleic acid	1330	10	
N-Propylamphetamine	1330	19	

(Continued on p. 84)

TABLE 4 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Salicylic acid	1330	19	10
Nicotine	1340	19	7, 10
Phenelzine	1340	This study	10
Cypenamine	1345	7	
Ephedrine	1350	19	7, 10, 15
Pseudoephedrine	1350	7	
Ectylurea	1360	19	10
Ethinamate	1360	19	10
Methoxyphenamine	1360	7, 10	
Acetanilide	1365	10	
p-Hydroxyphenylpyruvic acid	1380	10	
Ametazole	1390	This study	
Bemegride	1390	10	
Methylephedrine	1400	7, 10	
n-Caproic acid	1410	10	
Pheniprazine	1410	10	
Modaline	1420	7	
Phenmetrazine	1430	19	7, 10
Phendimetrazine	1440	19	7, 10, 15
Quinoline	1440	10	
Isoquinoline	1440	10	
Pheneturide	1450	15	
Etafedrine	1460	7, 15	
Nicotinamide	1460	10	
Salicylamide	1460	19	10
Warfarin	1460	19	10, 15
Phenoxypropazine	1465	7	
MDA (Methylenedioxyamphetamine)	1470	19	10
Metharbitone	1470	10	15
Sulphonal	1475	15	
Diethylpropion	1480	19	7, 10
Capric acid	1485	10	
Theophylline	1485	10	
Cantharidin	1490	19	
Carbaryl	1490	19	
Barbitone	1495	19	10, 15
Carbromal	1500	19	
Histamine	1500	10	
Mandelic acid	1500	10	
Nialamide	1500*	This study	
Nikethamide	1500	19	7, 10
Protokylol	1500	10	
Thiosalicylic acid	1500	10	
Methyprylone	1505	19	10, 15
Chlorothymol	1510	10	
2,4-Dinitrophenol	1510	10	
Methocarbamol	1510	10	
Phenprobamate	1520	15	
Benzocaine	1530	This study	8, 10
Hydrallazine	1530	10	
Leptazol	1535	This study	7, 10

TABLE 4 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
3,4-Dimethoxyphenethylamine	1540	19	
Mephenesin	1550	10	
Methimazole	1550	15	
Probarbital	1550	19	10, 15
Tolazoline	1550	15	
Clofibrate	1560	10	
Mephenesin carbamate	1570	10	
Iproniazid	1580	This study	10
Hydrastinine	1590	19	
Metronidazole	1590	10, 15	
Methsuximide	1595	19	
Allobarbitone	1600	10	15
Lauric acid	1600	10	
Nifenazone	1600	15	
Resorcinol	1610	10	
Aprobarbitone	1620	10	15
4,6-Dinitro-*o*-cresol	1620	10	
STP (Methyldimethoxyamphetamine)	1620	19	10
Zoxazolamine	1625	10	
Isoniazid	1630	19	10
Amylocaine	1635	8, 10	
Dyclonine	1640	10	
Phensuximide	1640	10	
Tolazamide	1650	10	
Butabarbitone	1655	10	
p-Nitromethylamphetamine	1655	10	
Butobarbitone	1660	10	15
Phenacetin	1660	19	8, 10, 15
Allylbarbituric acid	1670	10	15
Carbimazole	1670	15	
Cotinine	1670	8	
Styramate	1670	10	
Chlorphenesin carbamate	1690	10	
Carminic acid	1690	10	
Mescaline	1690	19	10, 15
5-Propyl-5-isobutylbarbituric acid	1690	10	
Tolbutamide	1690	10	
Talbutal	1700	10	15
Tybamate	1700	10	
Chlorzoxazone	1710	10, 15	
Paracetamol	1710	19	10
Isoprenaline	1720	10	
Methoxamine	1720	10, 15	
Amylobarbitone	1725	19	10, 15
Hippuric acid	1730	10	
Butethamate	1740	15	
Chlorpropamide	1740	10, 15	
Hydroxyphenamate	1740	19	10, 15
Lindane	1740	10	

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TABLE 4 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Meclofenoxate	1740	15	
Myristic acid	1740	10	
Pentachlorophenol	1740	This study	10
Pethidine	1740	19	8, 10, 15
Phenylsalicylate	1740	10	
Properidine	1740	15	
Tryptamine	1740	8, 10, 15, 19	
Norpethidine	1745	19	8
Dimethyltryptamine	1750	10, 15	
Pentobarbitone	1750	19	10, 15
Vinbarbitone	1750	10, 15	
Diazinon	1760	10	
Methohexitone	1760	10	
Fenmetramide	1765	8	
Pachycarpine	1765	19	
Nicoumalone	1770	10	
Sparteine	1770	10, 15	
Quinalbarbitone	1775	19	10, 15
Cotarnine	1780	19	
Lobeline	1780	19	
Methylphenidate	1780	19	7, 10
Phenanthrene	1780	10	
Secbutobarbitone	1780	19	
Anthracene	1790	10	
Betaprodine	1790	15	
Meprobamate	1790	19	8, 10, 15
Methoin	1795	19	10
Ethotoin	1800	10	
Pheniramine	1805	5	8, 15
Caffeine	1810	19	8, 10, 15
Physostigmine	1810	19	10
Glutethimide	1825	19	8, 10, 15
Betameprodine	1830	15	
Ketamine	1830	10, 15	
Phenazone	1830	19	10
Piperoxan	1830	15	
Thymoxamine	1830	15	
Trimeperidine	1830	15	
Alphameprodine	1840	15	
Dimenhydrinate	1840	10	
Theobromine	1840	10	
Ethoheptazine	1845	19	8, 10, 15
Prilocaine	1845	8, 10	
Benzphetamine	1850	7, 10	
Carisoprodol	1850	19	10
Etryptamine	1850	8	
Hexobarbitone	1850	10	
Thiopentone	1850	19	10
Tymazoline	1850	8	
Diphenhydramine	1855	19	8, 10
Lachesine	1860	15	

TABLE 4 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Lignocaine	1860	19	8, 10, 15
Mebutamate	1865	19	10
Dimethylthiambutene	1870	15	
Phencyclidine	1870	19	10, 15
Butylphthalate	1880	10	
Amidopyrine	1890	19	8, 10
Thiamylal	1890	19	10
Alphaprodine	1895	10, 15	
Acenocoumarin	1900	10	
Cyclandelate	1900	10	
Diethyltryptamine	1900	10	
Malathion	1900	10	
Methylphenobarbitone	1920	19	15
Xylometazoline	1920	10, 15	
Azapetine	1925	15	
Doxylamine	1925	10, 15	
Ethylmethylthiambutene	1925	15	
Orphenadrine	1925	5	8, 10, 15
Parathion	1925	10	
Phenyltoloxamine	1925	10, 15	
Hydroxyamylobarbitone	1930	15	
Aldrin	1950	19	
Ampyrone	1950	19	
Caramiphen	1950	15	
Cyclobarbitone	1950	10	15
Hexylcaine	1950	15	
Phenobarbitone	1950	19	10, 15
Piperocaine	1955	This study	10
Isocarboxazid	1960	19	10
Nystatin	1960	10	
Tetrahydrozoline	1960	8	
Tripelennamine	1960	19	8, 10, 15
Methapyrilene	1970	19	8, 15
Ethomoxane	1975	15	
Palmitic acid	1975	10	
Methaphenilene	1980	10	
Procarbazine	1990	10	
Procaine	1995	19	8, 10, 15
Bufotenine	2000	15	
Chlorpheniramine	2000	19	8, 10, 15
Diethylthiambutene	2000	15	
Eucatropine	2000	15	
Phenyramidol	2000	This study	10
Pyridoxamine	2000	19	
Amprotropine	2010	15	
Butanilicaine	2010	15	
Cyclizine	2010	19	8, 10, 15
Cyclopentolate	2010	15	
Ketobemidone	2010	15	

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TABLE 4 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Phenothiazine	2010	10, 15	
Pilocarpine	2010	10	15
Pipazethate	2010	15	
Thenyldiamine	2010	This study	10
Thiabendazole	2010	10	
Chlordane	2020	19	
Amisometradine	2025	10	
Apoatropine	2025	15	
Butallylonal	2025	10	
Hydroxypethidine	2025	15	
Dimethoxanate	2030	This study	
Methadone (cyclic metabolite)	2030	8	
Chlorphenoxamine	2040	10, 15	
Homatropine	2045	19	10, 15
Butethamine	2050	This study	10, 15
Carbinoxamine	2050	5	10, 15
Chlorbenside	2050	19	
Isopropamide	2060	10	
Brompheniramine	2070	10, 15	
Chlophedianol	2070	15	
Mepivacaine	2075	This study	10, 15
Normethadone	2080	8	
Diphenylpyraline	2090	This study	10, 15
Naphazoline	2090	This study	10
Dicyclomine	2100	This study	10
Heptabarbitone	2100	10	
Quinuronium	2100	15	
Dieldrin	2110	19	15
Dextromethorphan	2115	8, 15	
Procyclidine	2115	This study	10, 15
Mephenoxalone	2120	19	10
Isomethadone	2125	8	
Oxypertine	2125	15	
Chloropyrilene	2130	This study	10
Pyrimethamine	2140	19	10
Tacrine	2140	15	
Propranolol	2145	10, 15	
Thiantoin	2145	19	
Alphamethadol	2150	15	
Bromodiphenhydramine	2150	19	10, 15
Dopamine	2150	15	
Embramine	2150	15	
Pipradrol	2150	19	8, 10, 15
Butriptyline	2155	15	
Alphacetylmethadol	2160	15	
Phenindamine	2160	This study	8, 10, 15
Santonin	2160	10, 15	
Methadone	2170	19	8, 10, 15
Oxymetazoline	2170	10, 15	
Atropine	2175	19	10, 15
Fluopromazine	2175	15	

TABLE 4 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Linolenic acid	2175	10	
Stearic acid	2175	10	
Cocaine	2180	19	8, 10
Dextropropoxyphene	2180	19	8, 10, 15
Ergocryptine	2180	10	
Metaxalone	2180	10	
Methaqualone	2180	10, 15	8
Mefenamic acid	2185	10, 15	
Adiphenine	2190	19	10
Amitriptyline	2200	19	8, 10, 15
Chloroprocaine	2200	10, 15	
Perphenazine	2200	19	
Pipobroman	2200	10	
Thonzylamine	2200	This study	10, 15
Trimipramine	2200	15	
Mepyramine	2205	5	8, 10, 15
Picrotoxin	2205	19	
Amolanone	2210	10, 15	
Azacyclonol	2210	19	10, 15
Triflupromazine	2210	19	8, 10
Amethocaine	2215	19	10, 15
Chlorcyclizine	2215	19	8, 10, 15
Nortriptyline	2215	5	10, 15
Imipramine	2220	19	8, 10, 15
Cyclomethycaine	2225	10	
Hyoscyamine	2225	19	10
Levorphanol	2225	10, 15	
Metabutoxycaine	2225	10	
Benactyzine	2230	19	10
Benzhexol	2230	19	10, 15
Levomethorphan	2230	15	
Phenoxybenzamine	2230	10, 15	
Procainamide	2230	10, 15	
Protriptyline	2230	15	
Carbetapentane	2240	This study	10
Chlormezanone	2250	10	
Primidone	2250	19	8, 10, 15
Triprolidine	2250	19	8, 10, 15
Desipramine	2260	This study	10, 15
Isothipendyl	2260	This study	8, 10, 15
Promethazine	2260	19	8, 10, 15
Pentazocine	2265	19	8, 10, 15
Bupivacaine	2270	8	15
Dimethindene	2270	10, 15, 19	
Desomorphine	2275	15	
Aconitine	2280	19	
Biperiden	2280	15	
Diethazine	2280	15	
Harmine	2280	This study	10

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TABLE 4 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Anisinidione	2285	10	
Hyoscine	2285	19	10, 15
Carbamazepine	2290	15	
Dicophane	2290	19	10, 15
Methyldesorphine	2290	15	
Promazine	2295	19	8, 10, 15
Chlordiazepoxide	2300*	This study	15
Stilboestrol	2300	This study	10, 15
Pramoxine	2305	This study	10, 15
Dihydroergotamine	2310	19	
Etoxeridine	2310	15	
Benztropine	2320	10, 15	
Buphenine	2320	This study	
Primaquine	2320	This study	10
Probenecid	2320	This study	10
Propoxycaine	2320	15	
Trimeprazine	2320	This study	8, 10, 15
Piperidolate	2325	This study	10, 15
Antazoline	2330	19	8, 10, 15
Prothipendyl	2330	This study	10, 15
Oxazepam	2335	10, 15	
Phenytoin	2335	15, 19	
Dexoxadrol	2340	8	
Levallorphan	2340	5	10, 15
Phenazopyridine	2345	10	
Ethopropazine	2350	This study	10, 15
Gentisic acid	2350	10	
Propantheline	2350	10	
Cyproheptadine	2355	10, 15	
Ergotamine	2360	10	
Dihydrocodeine	2365	8	10, 15
Phenylbutazone	2370	10, 15	
Methyldihydromorphine	2375	15	
Metopon	2375	15	
Pholcodine	2380	10	
Codeine	2385	19	8, 10, 15
Hexoestrol	2400	10	
Diazepam	2410	5	10, 15
Methoxychlor	2410	19	10
Ethylmorphine	2415	19	10, 15
Dihydrocodeinone	2425	10	
Hydrocodone	2425	15	
Oxycodone	2425	15	
Pyrrobutamine	2430	19	8, 10, 15
Morphine	2435	19	8, 10, 15
Chlorpromazine	2440	19	8, 10, 15
Dihydromorphine	2440	15	
Xenysalate	2440	15	
Acetyldihydrocodeine	2450	15	
Mebhydrolin	2450	15	
Ethylisobutrazine	2455	8	

TABLE 4 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Tetrahydrocannabinol	2455	19	
Chlordiazepoxide	2460*	This study	
Butacaine	2470	5	10
Dipipanone	2470	5	8, 15
Methdilazine	2470	8, 10, 15	
Pipethanate	2470	10	
Desmethylchlorpromazine	2480	8	
Dibenzepin	2480	10	
Didesmethylchlorpromazine	2480	8	
6-Monoacetylmorphine	2480	15	
Hydromorphone	2490	10	
Methixene	2490	10	
Chlordiazepoxide	2500*	This study	10, 15
Ergocristine	2500	10	
Methoxypromazine	2500	19	10
Morpheridine	2500	15	
Thebacon	2500	15	
Chlorprothixene	2510	10	
Phenadoxone	2510	8	15
Methotrimeprazine	2515	This study	8, 10, 15
Norethynodrel	2520	10	
Oxymorphone	2520	10, 15	
Pyrathiazine	2520	8	10
Thebaine	2525	19	10
Oxyphencyclimine	2540	10	
Pentaquin	2540	15	
Prenylamine	2540	15	
Ethoxzolamide	2550	10	
Pecazine	2550	19	10, 15
Nalorphine	2570	19	10, 15
Chloroquine	2575	19	8, 15
Cinchonine	2575	19	8, 10, 15
Deptropine	2590	15	
Methyltestosterone	2610	15	
Trimethoprim	2610	15	
Diamorphine	2615	19	8, 10, 15
Phenacaine	2615	10	
Cinchonidine	2625	19	8, 15
Acepromazine	2665	8, 10, 15	
Phenazocine	2670	8, 10	
Nitrazepam	2675	5	15
Benzethidine	2680	15	
Clemizole	2680	5	10, 15
Trifluoperazine	2680	This study	8, 10, 15
Cinchocaine	2690	19	10, 15
Indomethacin	2690	10	
Fentanyl	2700	15	
Gallamine	2700	15	
Ethinyloestradiol	2710	10	

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TABLE 4 *(continued)*

Drug	*Retention index*	*Reference*	
		Source	*Check*
Propiomazine	2725	This study	15
Quinine	2755	19	10, 15
Quinidine	2760	19	10, 15
Captodiame	2775	19	10, 15
Hydroxyquinidine	2780	15	
Progesterone	2785	10	
Chlordiazepoxide	2790	This study	19
Hexachlorophane	2795	10, 15	
Ethoxyquin	2800	19	
Papaverine	2805	5	10, 15
Hydroxyzine	2840	19	10, 15
Anileridine	2845	19	10, 15
Dapsone	2860	19	10
Hydroxychloroquine	2860	19	10
Doxapram	2875	15	
Sanguinarine	2880	19	
Diphenadione	2910	10	
Prochlorperazine	2935	10	
Haloperidol	2940	10	
Dexamethasone	2950	10	
Hydrastine	2975	19	10
Cholesterol	3015	10	
Thiothixene	3015	10	
Strychnine	3040	19	10, 15
Fluphenazine	3045	19	
Meclozine	3050	19	10, 15
Methysergide	3050	10	
Triamcinolone	3070	10	
Noscapine	3100	10	
Thioridazine	3110	19	10
Rotenone	3250	10	
Pipamazine	3260	This study	
Thiethylperazine	3260	10	
Brucine	3280	10	
Buclizine	3285	19	10
Yohimbine	3290	19	10
Lysergide	3445	19	
Thiopropazate	3450	This study	
Carphenazine	3590	19	

* Major decomposition product.

because of decomposition, the retention indices are much less reproducible and will decrease with increased amount of drug injected onto the column. A suitable factor to use for routine identification purposes is $\pm$ 50 retention index units when more than 99% of experimentally determined values will fall within this range from the mean value. An experienced analyst using isothermal conditions and obtaining good symmetrical chromatographic peaks will obviously be able to obtain better reproducibility and it is expected that most values obtained will fall in the range $\pm$ 20 from

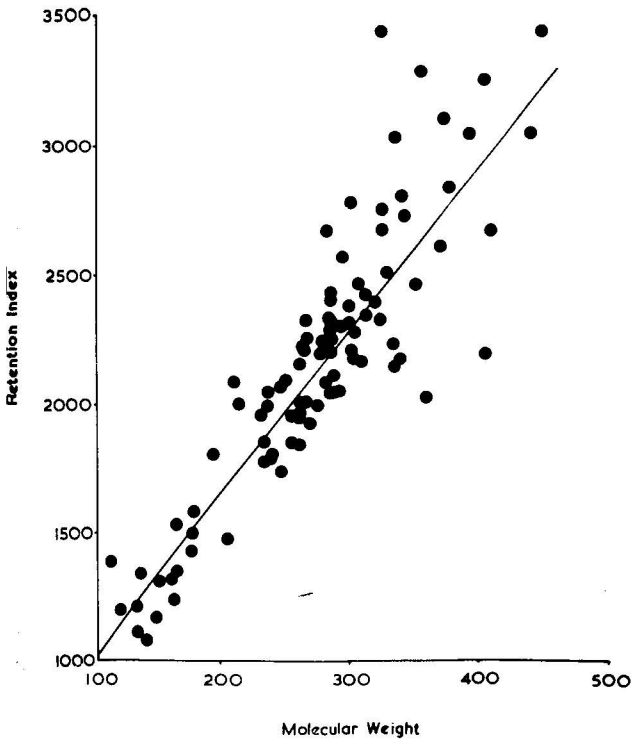

Fig. 2. Correlation of retention index and molecular weight for drugs chromatographed on an SE-30 column.

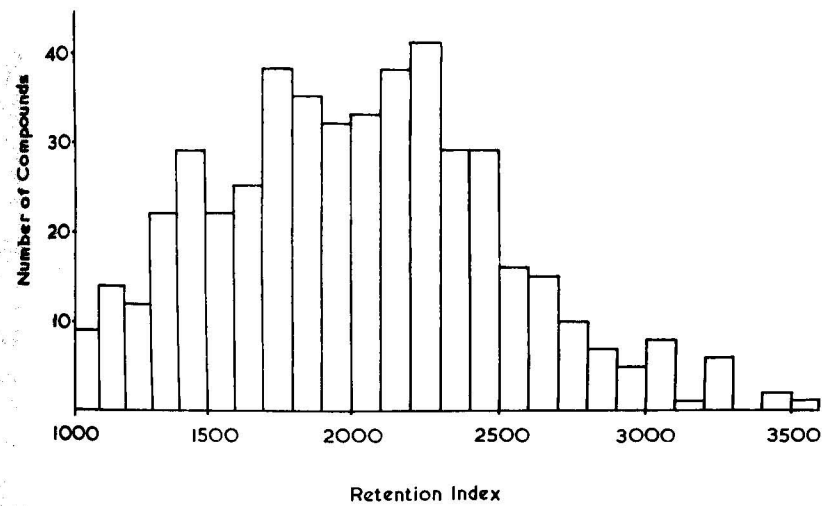

Fig. 3. Frequency histogram of retention indices of 478 drugs on an SE-30 column.

the values in Tables 3 and 4. It must be remembered that since the retention indices in Tables 3 and 4 were obtained from many sources, and each value is subject to some error, a drug with a smaller index than another may not necessarily be eluted first. For example, acetylsalicylic acid and phenylpropanolamine are quoted as having retention indices of 1295 and 1310, respectively, but under some conditions they may elute in the reverse order. The important feature to recognise is that they elute within the error factor of the value given in Tables 3 and 4.

Fig. 3 shows the histogram for the 478 drugs that gave peaks (two were not eluted between 1000 and 3500). Obviously, if a peak is obtained with a retention index of 2200, this information is far less useful for identification purposes than a value of 3500, where fewer compounds are possible identities. Thus, Fig. 3 may help in determining the usefulness of the retention index for an unknown drug for identification purposes.

It is hoped that by creating this unified collection of data for the GLC identification of drugs by measurement of their retention indices on an SE-30 column it will aid analysts to standardise on this single "preferred liquid phase" and that future analysis will be performed using this phase.

4. ACKNOWLEDGEMENT

The author is indebted to those scientists in the Home Office Forensic Science Laboratories whose help enabled this project to be completed.

5. SUMMARY

The dimethyl silicone elastomer SE-30 has been chosen as the preferred liquid phase for the gas–liquid chromatographic analysis of drugs, and retention index data have been compiled for 480 drugs and commonly occurring chemicals such as plasticisers. The inter-laboratory variation in measurement of retention indices has been measured for three drugs in eleven laboratories and the standard deviations were between 20 and 15 retention index units.

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Journal of Chromatography, 113 (1975) 97–137
Chromatographic Reviews

CHREV. 82

CHROMATOGRAPHIC ANALYSIS OF FUNGICIDES

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(Received July 19th, 1974)

CONTENTS

1. INTRODUCTION

The term "pesticide" (economic poison) is generally taken to include those compounds functioning as insecticides, nematocides, miticides (acaracides), growth regulators, fungicides, and rodenticides. Fungicides are compounds used on farm crops as protective agents against the attack of a fungus. Types of fungicides in use today include soil fungicides, seed protectants, foliage and fruit protectants, and eradicants.

This review will cover chromatographic methods for the separation and analysis of fungicides and metabolites as standards and as residues in various sample matrices. Only organic fungicides will be included, and these will be classified as organomercury compounds, carbon disulfide–amine reaction products (dithiocarbamates), halogen-containing compounds, cationic compounds, and miscellaneous compounds. The difficulty with neat classification of fungicides is indicated by the fact that within some of these classes are compounds of different chemical types, *e.g.*, Dyrene and dichlone both contain halogen but the former is a triazine and the latter a quinone. Some of the newer organic fungicides are "systemic" in nature, that is they are translocated to other parts of the plant than those to which they are applied. However, studies on the mechanism of fungicide action will not be covered in this review, nor will properties, uses, ecological, or toxicological aspects. The analysis of fungicide formulations will be only occasionally mentioned. Formulae and alternate names for all fungicides mentioned will be found in the appendix at the end of this paper.

The literature of fungicide chromatography is covered through May, 1974. The attempt was made to be selective in the choice of cited references and to present only those methods which appear most likely to give satisfactory results in practical analytical situations.

Analytical methods for pesticides usually consist of the following steps: sampling and sample preparation, extraction, liquid–liquid partition cleanup, liquid–solid cleanup, and determinative procedures. The exact nature of the procedures required depends upon the residue of interest and the sample type. Tentative qualitative identification and quantitation of residues is normally carried out using either gas–liquid chromatography (GLC), thin-layer chromatography (TLC) or high-speed liquid chromatography (HSLC), while confirmation of identity is obtained by combination of two or more chromatographic methods, chemical derivatization, or spectrometric analysis. General aspects of chromatographic pesticide residue analysis have been covered in two recent books[1,2] and a review article[3], while other review articles have dealt with the chromatography of herbicides[4], carbamate insecticides and herbicides[5,6], triazine herbicides[7], fumigants[8], and organomercurials[9].

The primary chromatographic method presently employed in pesticide residue analysis is gas–liquid partition chromatography. This is because of the availability of a series of sensitive and selective detectors[10,11] for pesticide compounds containing halogen, P, S, and N atoms. These detectors, which are applicable in fungicide analyses, include the relatively non-specific electron capture detector, and the specific microcoulometric, flame photometric, alkali flame ionization, and electrolytic conductivity detectors. The latter greatly facilitate analysis due to the minimal amount of cleanup of extracts that is required. Cochrane and co-workers have systematically

evaluated and compared these detectors for pesticide analysis in a valuable series of papers[12–16]. Thermal conductivity and flame ionization detectors are normally not useful for residue analysis but may be employed to advantage for pesticide formulation analysis.

2. BEHAVIOR OF FUNGICIDES IN MULTIRESIDUE PESTICIDE ANALYTICAL PROCEDURES

Various systematic schemes have been developed for analyzing residues of a large number of pesticides. Although chlorinated and organophosphorus insecticides and chlorophenoxy acid herbicides have mostly been studied, some fungicides and metabolites have been tested through various portions of these multiresidue procedures. The fungicides PCNB and TCNB were quantitatively extracted (80–100%) from fruits, vegetables, and cereal grains by blending with acetonitrile[17,18]. Fungicides recovered from evaporated acetonitrile extracts by hexane partition included captan, chlorothalonil, Dyrene, folpet, and PCNB[17,18]. Chlorothalonil, captan, HCB, and PCNB were recovered when an acetonitrile extract was diluted with water, sodium sulfate solution was added, and some dichloromethane was added to the hexane before partitioning. A mildly acid buffer was required to prevent hydrolytic decomposition of captan[18]. Dyrene and PCNB were recovered from hexane extracts of butterfat by acetonitrile partition[18].

Pesticides extracted from samples by acetonitrile and then partitioned into hexane may be cleaned up prior to GLC or TLC by chromatography on a 3.0-g Darco G60 carbon-Solka Floc BW40 cellulose column contained in a 100-ml buret. Captan and PCNB are eluted in fraction 1 with 180 ml 1.5% acetonitrile in hexane, folpet with 200 ml chloroform and chlorothalonil and Dyrene with 200 ml benzene[17,18]. Fraction 1 will also contain most chlorinated insecticides (DDT) and polychlorinated biphenyls (PCBs), while fraction 2 will contain organophosphorus insecticides (parathion).

A differential elution scheme was worked out for separating and confirming some 55 pesticides by use of a 15 × 2.5 cm column of Florisil partially deactivated with 2% water and eluted in turn with ten 300-ml portions of hexane containing 0–30% dichloromethane followed by 5–30% ethyl acetate. Six fungicides were included in the study[18] and were eluted as follows: HCB in the hexane fraction, PCNB and TCNB in the two fractions of hexane containing 5 and 10% dichloromethane, Dyrene in the 5% ethyl acetate fraction, chlorothalonil in the 5 and 10% ethyl acetate fractions, and captan in the 20% ethyl acetate fraction. All recoveries were greater than 95%, except captan, which was 80%. The United States FDA multiresidue Florisil cleanup procedure[19] employs activated Florisil columns (4 in. × 22 mm), eluted with light petroleum (b.p. 30–60°C) containing 6–65% diethyl ether. Table 1 shows results for the fungicides tested through this procedure. With an improved elution system, PCNB and TCNB were eluted > 90% from the FDA Florisil column with 200 ml 20% dichloromethane in hexane, DCNA and dichlone with 200 ml 50% dichloromethane–0.35% acetonitrile–49.65% hexane, and captafol and captan with 200 ml 50% dichloromethane–1.5% acetonitrile–48.5% hexane. Folpet divided between the second and third fractions[20].

TABLE 1

ELUTION PATTERN OF FUNGICIDES FROM AN ACTIVATED (1200°F) FLORISIL COLUMN[18,19]

Fungicide	*Diethyl ether (%) in light petroleum (b.p. 30–60 °C) used for elution (200-ml fractions)*	*Recovery (%)*
DCNA (Botran)	6, 15*; 6, 15, 20	<80; >80
Captafol	6, 15	0
Captan	6, 15	0
Captan epoxide	6, 15	0
Chloroneb	6	>80
Chlorothalonil	6, 15	0
Dichlone	6, 15	0
Dinocap	6, 15	>80
Dyrene	6, 15	>80
Folpet	6, 15, 20	>80
Hexachlorobenzene	6	60**; >80***
Hexachlorophene	6, 15, 50	0
Chloronitropropane (Korax)	6, 15	0
PCNB	6	>80
TCNB	6	>80
Tetraiodoethylene	6	>80

* 6, 15 means 6% followed by 15%.
** Fatty foods.
*** Non-fatty foods.

Captan and HCB were recovered > 80% at levels greater than 0.05 ppm from spiked plant and animal tissues and mixtures by a low-temperature (−78°C) precipitation cleanup method used to separate fats, oils, and water from acetone–benzene–1 *N* H_2SO_4 (19:1:1) extracts[18,21].

Another important multiresidue scheme in use today employs deactivated (5% water) silica columns for separation and cleanup of residues prior to GC[22–24]. Elution is two-stage, the first eluent being hexane and the second diethyl ether–hexane (1:9). This system has been used mainly for organochlorine insecticides, PCBs, and a few organophosphate insecticides. Although no fungicide recoveries have been reported, it is likely that many would be quantitatately eluted, especially if a third, more polar solvent were used.

Only a few types of GLC liquid phases have been extensively used for separating pesticide mixtures, and the relative retention times of some fungicides on these phases are shown in Table 2. Temperature and carrier gas flow-rates are chosen for routine analyses with these columns so that the reference compound elutes in approximately the time given. Column temperatures of 180–225°C and flow-rates of 60–120 ml/min are typical for 6-ft. × ¼-in. columns in which the liquid phases are coated on the support (*e.g.*, Chromosorb W AW) at levels of 3–15%.

TLC studies of a series of organochlorine pesticides on 250-μm layers of MN silica gel G-HR included the fungicides chlorothalonil and captan[18]. The pesticides were detected by spraying with the usual $AgNO_3$–2-phenoxyethanol reagent solution followed by exposure to ultraviolet (UV) light. Chlorothalonil had an R_F value of 0.3 when developed with 1% acetone in hexane, while captan remained at the origin

TABLE 2

RETENTION TIMES OF FUNGICIDES RELATIVE TO PARATHION ON SEVERAL GLC LIQUID PHASES[18,19,25]

Compound	RRT_p			
	*NP**	*IP***	*P****	*M*§
TCNB	28	—	—	20
HCB	43	10	—	22
PCNB	50	—	—	32
Chlorothalonil	52	60	77	64
Tetraiodoethylene	54	—	—	27
Dichlone	54	—	—	46
Parathion	100 (5 min)	100 (20 min)	100 (4 min)	100 (14 min)
Dyrene	120	50	182	79, 96§§, 133§§
Folpet	120	101	—	107
Captan	122	40	—	112
Hexachlorophene	—	130	—	—

* Non-polar phases, *e.g.* DC-11, DC-200, SE-30, OV-1.
** Intermediate polarity phases, *e.g.* OV-210, QF-1.
*** Polar phases, *e.g.* DEGS.
§ Mixed phases, *e.g.* SE-30/QF-1.
§§ Minor peak.

with this solvent and with hexane, benzene–hexane (1:1), and 1% methanol in hexane.

The above indicates the results obtained for a few fungicides which have been tested through parts of several widely used multiresidue pesticide screening procedures. Other fungicides, especially those which are apolar, might be successfully analyzed by these procedures, but in most cases their behavior has not been studied nor has their recovery been validated. The following sections of this review list more specific procedures related to the analysis of certain fungicide residues.

3. ORGANOMERCURY COMPOUNDS

The identification and determination of organomercurial fungicide residues by TLC and GLC of the dithizonates was reported by Tatton and Wagstaffe[26]. R_F values and retention times are shown in Table 3. The method for determination of residues of these fungicides at 0.01- to 5-ppm levels in various foodstuffs involved extraction of the sample with a slightly alkaline solution of cysteine hydrochloride in 2-propanol, washing of the extract with diethyl ether, and extraction of the organomercurials from the aqueous solution using 0.005% dithizone in diethyl ether. The extract was dried by passage through a Na_2SO_4 column, concentrated, and examined by TLC and/or GLC as described in Table 3. Gherardi *et al.*[27] also used dithizonates for the separation of methylmercury and phenylmercury by development on 0.3-mm alumina thin layers (activated at 150 °C for 30 min) with diethyl ether–light petroleum ether (3:7) as solvent. Dithizonates were identified by R_F values and spot colors. Mercury compounds were extracted by the Westöö[28] method from canned tuna fish samples prior to derivatization. Underivatized organic mercury halides were detected

TABLE 3

R_F VALUES × 100 OBTAINED BY TLC AND GLC RETENTION TIMES (min) OF ORGANOMERCURY FUNGICIDE DITHIZONATES[26]

Systems: (1) Silica gel/hexane–acetone (9:1). (2) silica gel/hexane–acetone (19:1). (3) silica gel/hexane–acetone (93:7). (4) alumina/hexane–acetone (19:1). Layer thickness, 250 μm; detection by natural yellow or red colors. (5) 2% PEGS on Chromosorb G AW DMCS, 60–80 mesh; glass column 1.5 m × 3 mm; nitrogen carrier gas; ECD. (6) Same as (5) except 1% PEGS and 1.2-m column length.

Dithizonate	*TLC systems*				*GLC systems*	
	1	*2*	*3*	*4*	*5*	*6*
Methylmercury	64	48	57	89	1.2	—
Ethylmercury	64	51	62	91	2.0	—
Methoxyethylmercury	32	16	25	58	4.9	—
Ethoxymethylmercury	44	23	34	71	4.9	—
Phenylmercury	48	34	46	72	27.0	5.0
Tolylmercury	52	40	53	79	19.5	3.2
Mercury di-dithizonate	19	9	17	19	—	—

by TLC on silica gel under similar conditions with 4,4′-bis(dimethylamino)thiobenzophenone as spray reagent.

Geike and Schuphan[29] studied the detection of organomercury fungicides after TLC by enzymatic and chemical techniques. Urease proved to be most sensitive for detection, with limits of 50–1000 ng for the fungicides and 1–60 μg for impurities found in the fungicides tested. Bovine liver esterase and α- and β-amylase were also inhibited by the fungicides, but detection was generally less sensitive. Chemical detection with sodium sulfide and dithizone was sensitive to 0.5–20 μg of the fungicides, and impurities did not interfere. Diisopropyl ether and chloroform–ethyl acetate (10:4) were suitable solvents for the separation of the nine fungicides studied, namely methylmercury chloride, methylmercury sulfate, methoxyethylmercury chloride, phenylmercury acetate, Merthiolate, Germisan, Quinex, Panogen, and Memmi.

Bache and coworkers[30,31] developed GLC methods for determining organomercuric fungicides at levels of 1 ppb* in crops grown in treated soils. A microwave powered plasma emission detector and columns and conditions as shown in Table 4 were employed.

Ealy *et al.*[32] determined methyl-, ethyl-, and methoxyethylmercury halides in environmental samples by leaching with molar sodium iodide for 24 h, extracting the alkylmercury iodides into benzene, and determination by GLC on a column of 5% cyclohexylenedimethanol succinate on Anakrom ABS at 200 °C with nitrogen carrier gas and an electron capture detector. Good separation of peaks was obtained for the mercury compounds as either chlorides, bromides, or iodides.

The GLC determination of inorganic mercury alone or in the presence of organomercurials (methyl-, ethyl-, and phenylmercury(II) chlorides) in water and a wide range of biological media was studied by Zarnegar and Mushak[33]. The method for inorganic mercury was based on the ability of various organometallics [*e.g.*, pentacyanomethylcobaltate(III)] to react electrophilically with the mercury to yield

* Throughout this article, the American billion (10^9) is meant.

TABLE 4

GAS CHROMATOGRAPHIC RETENTION TIMES AND SENSITIVITIES OF ORGANOMERCURY FUNGICIDES[31]

Compound	*Retention time (min)**	*Sensitivity (ng)***
Dimethylmercury	6.8	0.6
Methylmercury chloride	2.8	0.6
Methylmercury dicyanodiamide	2.8	0.8
Benzylmercury acetate	45.0	8.8
Methylmercury dithizonate	3.0	0.7

* Column for dimethylmercury: 2 ft., Chromosorb 101, 60–80 mesh, 100°C, 80 ml/min carrier gas flow-rate; for other compounds: 6 ft., 20% OV-17/QF-1 (1:1) on Gas-Chrom Q, 80–100 mesh, Carbowax treated, 152°C, 80 ml/min.

** For 50 % full scale deflection.

alkyl and aryl mercurials which were determined by GLC. Co-determination of inorganic mercury and the organomercurials originally present in the samples was carried out by sequential (difference) or simultaneous procedures. An electron capture chromatograph with 18-in. coiled-glass columns containing Durapak Carbowax 400 (low capacity factor, K') on Porasil F (140 or 170°C) or 10% DEGS on Anakrom SD (190°C) and argon–methane carrier gas proved optimal. Low nanogram levels of mercury were detected with this procedure.

4. DITHIOCARBAMATES

The compounds covered in this section include dimethyldithiocarbamates (ferbam, thiram, ziram), ethylenebisdithiocarbamates (nabam, maneb, zineb), the breakdown products ethylenethiourea and carbon disulfide, and dazomet.

The dithiocarbamates constitute the most important organic fungicides used to control plant fungus diseases. Residues of these compounds have traditionally been determined by a CS_2 evolution method with colorimetric read out[34–37]. Alternatively, ferbam, maneb, nabam, thiram, zineb, and ziram residues may be analyzed indirectly by GLC determination of the CS_2 generated when a fruit or vegetable sample and the residue are reacted with 1.5% $SnCl_2$ in 4 N HCl at 60°C in a closed system. An aliquot of head-space gas in the reaction flask is then injected into a chromatograph equipped with a flame photometric detector in the sulfur mode (394 nm) or an electron capture detector. Nanogram quantities of CS_2 may be detected, and fungicides at 3.5 and 7.0 ppm levels were recovered in the range 82–112% from a variety of spiked crops[18,38]. The column used for GLC of CS_2 was 6 ft. $\times \frac{1}{4}$ in., packed with 10% SE-30 or 6% QF-1/4% SE-30 on Chromosorb W HMDS at 50°C. With a carrier flow-rate of 40 ml/min, CS_2 eluted in about 1 min. The electron capture detector (ECD) gave a linear plot for peak height *vs.* nanograms CS_2 injected between 0.14 and 0.69 ng while the flame photometric detector (FPD) gave a linear logarithmic plot between 0.069 and 0.41 ng[18,38].

Zielinski and Fishbein[39] reported that zineb, maneb, and nabam released ethylenethiourea when fungicide samples were injected into a gas chromatograph.

A 6-ft. column of 4% QF-1 at 180°C and a nitrogen carrier gas flow-rate of 86 ml/min gave a retention time of 3.75 min for the thermal decomposition product.

The dithiocarbamates zineb, ziram, and ferbam have been "stripped" or washed from surfaces of some vegetable and fruit samples with chloroform prior to TLC analysis[18,40]. This procedure gave higher recoveries for some compounds compared with extraction by blending. Benzene (A) was the solvent for dimethyldithiocarbamates and acetic acid–methanol–benzene (1:2:12) (B) for ethylenebisdithiocarbamates on silica gel thin layers. Spots were detected with a 2.5-μg lower limit by spraying with cupric chloride–hydroxylamine hydrochloride chromogenic reagent to produce yellow, green, or brown colors. R_F values were as follows[18,41]:

Compound	*Solvent A*	*Solvent B*
Thiram	0.17	0.86
Tetramethylthiuram	0.30	—
Ziram	0.68	0.94
Maneb	0.98	0.98
Zineb	0.95	0.88

The paper chromatography (PC) of ferbam, maneb, nabam, thiram, zineb, and ziram was studied using formamide-impregnated paper developed with chloroform, petroleum ether, or chloroform–hexane (1:4). Reagents for detection included sodium azide–iodine, zincon, and 4-chlororesorcinol + ammonia. These systems allowed separation and identification of individual compounds[42]. Weltzien[43] employed ascending PC with *n*-butanol–acetic acid–water (4:1:1) solvent and bioautographic detection for separation and detection of thiram (0.81; 0.1), Polyram (0.83; 2.0), ziram (0.80; 0.1), ferbam (0.81; 0.1), zineb (streak; 5.0); Urbazit (0.83; 0.1), Brestan (0.91; 2.5), captan (0.89; 2.0), copper oxychloride (0.22; 5.0), Ceresan (0.80; 25), and Cerenox (0.78; 10). R_F values and sensitivities in μg are given in the parentheses.

Vekshtein and Klisenko[44] separated dialkyldithiocarbamates and metabolites on alumina layers with heptane–benzene–acetone (10:1:22.5) solvent, spraying with iodide–azide reagent for detection. Spots were eluted from the layer with 0.2 *N* NaOH and analyzed by UV spectrophotometry at 250–280 nm. Determinations in plant and animal material were made at the 0.02 to 0.1-ppm level by extraction of samples with chloroform (or chloroform + 0.5% NaOH for acid samples) and two-dimensional TLC with carbon tetrachloride–*n*-butanol (100:0.75) as solvent for cleanup. Kosmatyi *et al.*[45] determined zineb in tobacco plants (10 μg/100 g) by a TLC method based on acid decomposition of the fungicide, absorption of the CS_2 produced in methanolic KOH, and chromatography of the resulting methylxanthate on silica gel KSK–alumina (1:1) with acetone–methanol (20:1). The chromogenic reagent was 2% $(NH_4)_2MoO_4$, acidified with HCl, and the size of the spot was measured by densitometry.

Porter[46] used TLC to detect the presence of thiram in wheat seeds. Chloroform seed washings and standards were spotted on silica gel layers and developed with chloroform–carbon tetrachloride (3:1) in an S-chamber. Visualization of thiram was obtained by spraying the layer with starch until it was opalescent and then with sodium azide–starch solution to produce white spots on a blue-black background.

Up to 30 min was required for full color development. The R_F value of thiram was *ca.* 0.19–0.20 and the detection limit was 0.01 μg. Diameters of developed spots were used to estimate sample concentrations relative to standards, all initial zones being limited to 1 mm diameter in size. The separation of thiram, ziram, and zineb was carried out[47] on silica gel layers developed with acetone, respective R_F values being 0.53, 0.44, and 0.38. Reaction with iodine vapors was used for detection at unspecified levels.

Ethylenethiourea (ETU; 2-imidazolidinethione) is a potentially hazardous[48] degradation product of ethylenebisdithiocarbamate fungicides (nabam, maneb, zineb, Dithanes, etc.)[49], which has received wide attention over the past several years. ETU is also a contaminant in formulated EBDC fungicide products. Onley and Yip[50] and Yip *et al.*[51] determined ETU in fruits, vegetables, and milk at 0.02- to 10-ppm levels by extraction with an ethanol–chloroform mixture, cleanup on a cellulose column, and TLC after further cleanup on an aluminum oxide column eluted with methanol–acetonitrile–benzene (3:15:82) or GLC after derivatization with 1-bromobutane. TLC was carried out on alumina layers developed in saturated tanks with methanol–chloroform–benzene (1:5:10). ETU was detected with a sensitivity of 0.5 μg as a blue spot with $R_F \approx 0.23$ by spraying with Grote's reagent. GLC of the bromobutane derivative was carried out on a 6-ft. 30% DC-200/5% SE-30 column to the end of which was connected a 1-ft. anhydrous K_2CO_3 column, both at 200 °C. A KCl–$RbSO_4$ (1:1) thermionic detector[52] gave 50% full scale deflection for about 70 ng ETU derivative. The ETU derivative under the TLC conditions above gave a reddish-purple spot with $R_F \approx 0.43$.

ETU in commercial ethylenebisdithiocarbamate formulations was determined on a 3-ft. column of 2% Carbowax at 220°C with a thermal conductivity detector[53]. Newsome[54] determined ETU residues in apples (0.01–1 ppm) after conversion to the S-benzyl derivative followed by extraction, trifluoroacetylation, GLC-ECD, and confirmation by mass spectrometry. Blazquez[55] determined ETU residues in tomato foliage with a sensitivity of 1 ppm by a silica gel TLC method after extraction with dioxane. Two chromatographic solvents were used: chloroform–*n*-butanol–methanol–water (100:5:1:0.5) and dioxane–formaldehyde–acetic acid–water (3:1:1.5:1). Detection reagents used were iodine–starch and potassium ferricyanide–ferric chloride (1:1). Onley *et al.*[56] employed GLC-FPD (S mode) and sweep co-distillation cleanup for analysis of ETU in food crops. Cook and Leppert[57] determined ETU on potatoes (0.05 ppm for 60-g samples) using HSLC with UV detection after Florisil column and dichloromethane–water partition cleanup of methanol extracts. Haines and Adler[58] used methanol extraction, alumina column cleanup, derivatization with 1-bromobutane and GLC-FPD with a 6-ft. 20% SE-30 column at 200°C for determination of ETU at 0.01-ppm levels in food crops. The volatile derivative formed with ETU and bromobutane was characterized as 2-*n*-butylmercapto-2-imidazoline. Cruickshank and Jarrow[59] studied ETU degradation after UV irradiation on a silica gel thin layer. ETU and its photolysis products were separated with diethyl ether–methanol (9:1) (R_F ETU = 0.50), *n*-butanol–acetic acid–water (4:1:1) (0.71), 2-butanone–pyridine–water–acetic acid (70:15:15:2) (0.85), and benzene–ethyl acetate–diethylamine–methanol (50:40:10:8) (0.57). Detection was made by a combination of fluorescence quench, ninhydrin, and Ehrlich's reagent.

The fungicides dazomet and Vapam are similar to those already mentioned in

this section since they are manufactured from carbon disulfide and amines. Although no specific reports on their chromatographic analysis have been found, it is possible that they can be determined by a CS_2 evolution–GLC method similar to that described above.

5. CATIONIC COMPOUNDS

Two long-chain, nitrogen-containing cationic compounds are important agricultural fungicides, namely dodine and glyodin. No gas chromatographic procedures have been developed for these compounds, and residues are normally analyzed by colorimetric methods. The colorimetric method for dodine[60] involves formation of a complex with bromcresol purple, extraction of the complex into chloroform, and hydrolysis to form the colored product. Glyodin interferes with this method, so a qualitative test for distinguishing between the two fungicides on paper chromatograms was developed[61]. An aqueous solution containing 10% sodium hydroxide, 10% sodium nitroprusside, and 10% potassium ferricyanide was prepared, diluted with three volumes of water and allowed to stand until the color had changed to pale yellow. This reagent when sprayed on chromatograms gave a blue color with glyodin and a red color with dodine.

6. FUNGICIDES CONTAINING HALOGEN ATOMS

Kilgore and White[62] carried out separations of mixed chlorinated fungicides, isomers, and derivatives with both an all-glass isothermal system utilizing an ECD and an all-metal temperature-programmed system utilizing a flame ionization detector (FID). For isothermal separation, a 5% QF-1 column at 180°C was found superior to a 5% DC-11 column, but later peaks were diffuse and tailed. When the 5% QF-1 column was temperature programmed between 100 and 200°C (FID), a complete separation of chloronitropropane, PCP, HCB, TCNB, chloranil, PCNB, DCNA, dichlone, and Dyrene was achieved in about 25 min. Only the rear of the PCP peak exhibited any tailing. Fig. 1 shows the separation of a more complex mixture of seventeen fungicides, including isomers and derivatives, with this system.

Hutzinger *et al.*[63] recorded the 70-eV mass spectra of fifteen chlorinated aromatic fungicides. The characteristic patterns are useful for confirmation of residues of these fungicides tentatively identified by chromatography.

A. Captan, folpet, and captafol (see also Sections 6L and 6P)

These three phthalimide compounds are protective, non-systemic fungicides with closely related structures. Formulation analysis of captan and folpet was described by Crossley[64] using a GLC column of 3% XE-60 supported on Chromosorb G at 200°C with a thermal conductivity detector (TCD). Formulation analysis of captafol has been carried out on a column of 5% QF-1 on Chromosorb G at 260°C, also with a TC detector[65].

Pomerantz *et al.*[66] detected captan, folpet, and captafol residues in a variety of raw agricultural commodities at 0.1- to 2-ppm levels. The residues were extracted

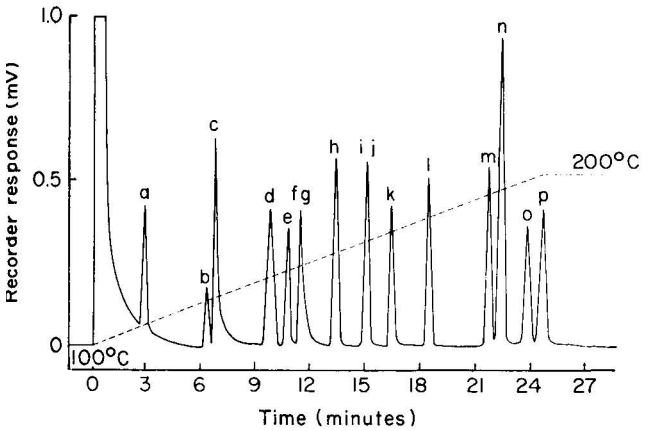

Fig. 1. Programmed-temperature separation of seventeen chlorinated fungicides, isomers, and derivatives on a stainless-steel column, 6 ft. × 1/8 in., containing 5% QF-1 on Chromosorb G (AW-DMCS), temperature programmed between 100 and 200°C at 4°C/min; helium carrier gas; FID; 1 μg of each compound. a = Chloronitropropane; b = 2,4,6-trichlorophenol, methyl ether; c = 2,4,5-trichlorophenol, 2,4,6-trichlorophenol; d = 2,4,5-trichlorophenol, methyl ether; e = 2,4,5,6-tetrachlorophenol, methyl ether; f = 2,4,5,6-tetrachlorophenol; g = PCP; h = HCB; i = PCP, methyl ether; j = TCNB; k = chloranil; l = PCNB; m = 1,2,4-trichloro-3,5-dinitrobenzene; n = dichlone; o = 1,2,3-trichloro-4,6-dinitrobenzene; p = Dyrene.

with acetonitrile, partitioned into dichloromethane–light petroleum (20:80) and purified by chromatography on a Florisil column eluted with dichloromethane–light petroleum (20:80) followed by dichloromethane–acetonitrile–light petroleum (50:1.5:48.5). The fractions were concentrated and analyzed by GLC on a stationary phase of 5% QF-1 on Chromosorb W HP at 155°C with 120 ml/min nitrogen flow-rate and an ECD. Captan and folpet were well separated, with the former eluting in about 12 min. Four nanograms of captan caused one-half full scale detector deflection. Kilgore *et al.*[67] determined captan residues (0.01 ppm) on fruits, vegetables, and cottonseed by GLC-ECD with a 10% DC-200 column at 185°C. Pomerantz and Ross[68] reported retention data for captan, folpet, captafol, and metabolites on 10% DC-200 and 15% QF-1/10% DC-200 (1:1) columns at 210° and 200°C, respectively. Captan residues present in cherries and raw agricultural products were recovered >80% at 1.6-ppm levels by extraction, charcoal cleanup[69], and GLC on a 10% DC-200 column with a Coulson conductivity detector.

Crossley[65] described the determination of captafol residues in crops on a 5% QF-1 GLC column at 190 °C with electron capture detection. When residues were above 5 ppm no cleanup was required, but below 5 ppm silica gel TLC was recommended for cleanup. Some oily crops required additional cleanup by solvent partitioning or Florisil column chromatography (benzene eluent) prior to TLC. The solvent for TLC was 2.5% diethyl ether in benzene, with captafol showing an R_F value of 0.4 after two ascending developments. Detection was with $KMnO_4$ reagent (see Table 5). Twenty-seven pesticides were tested, including captan and folpet, and did not interfere in the GLC step of the procedure. Presumably all three fungicides could be determined by this same overall method.

Baker and Flaherty[70] determined the three compounds simultaneously in

TABLE 5

R_F VALUES AND DETECTION TESTS FOR CAPTAN-TYPE COMPOUNDS[68]

Compound	R_F *relative to captan*	*Color response***			
		*1****	*2*§	*3*§§	*4*§§§
Captan	1.00*	Y/W (≤1)	Y/P (0.2)	—	
Folpet	1.14	Y/W (≤1)	Y/P (1)	—	
Captafol	1.02	—	Y/P (0.2)	OB/W (0.2)	
Captan epoxide	0.70	Y/W (≤1)	Y/P (1)	—	
Captafol epoxide	0.68	—	Y/P (1)	OB/W (0.2)	
Tetrahydrophthalimide	0.19	—	Y/P (0.5)	—	BG/Y (1)
Phthalimide	0.35	—	—	—	BG/Y (1–2)
Tetrahydrophthalimide epoxide	0.08	—	—	—	BG/Y (1–2)

* R_F of captan was 0.61, 154-mm solvent front distance.

** Y/W = yellow spot on white background; Y/P = yellow spot on pink background; OB/W = orange-brown spot on white background; BG/Y = blue-green spot on yellow background; — = no color development. Lower limit of detection in μg given in parentheses.

*** 15 g resorcinol dissolved in glacial acetic acid and diluted to 100 ml; spray and heat for 10–15 min at 110 °C.

§ 1 ml aqueous $KMnO_4$ (1.5 g/25 ml) diluted with acetone to 100 ml.

§§ 0.25 g DMPD dihydrochloride in methanolic NaOH (0.7 g NaOH in 50 ml absolute methanol).

§§§ 10 g $K_2Cr_2O_7$ in 80 ml conc. H_2SO_4 diluted to 200 ml with water; spray and heat for 20–30 min at 130 °C.

fruits at 0.1- to 1-ppm levels by a GLC-ECD method employing a 3% XE-60 phase on Chromosorb W at 190°C. This same column was earlier used by Bevenue and Ogata[71] to separate captan and folpet residues extracted from fresh papayas. Relative retention times on this column as reported by Baker and Flaherty[70] were captan 1.00, folpet 0.82, and captafol 3.34. Several chlorinated and organophosphorous insecticides had similar retention times to captan and folpet on this column, so a 1% SE-52 liquid phase on Chromosorb W at 180 °C was used to separate captan and folpet from these insecticides and to confirm the presence of the fungicides. Residues were initially extracted into acetonitrile, partitioned into hexane–diethyl ether (1:1), and cleaned up on an activated silica gel column eluted with hexane–diethyl ether (1:1). To remove any interfering insecticides prior to GLC, the silica gel column was first washed with hexane–diethyl ether (9:1) to elute the insecticides and then pure diethyl ether to elute the fungicides.

Archer and Corbin[72] detected captan residues at 1-μg levels in prune fruits and blossoms in the presence of captafol by TLC. The residue, after benzene extraction and Florisil cleanup, was spotted on a silica gel H plate and developed with isopropanol–benzene (4:96). Spraying with either resorcinol–glacial acetic acid or pyridine tetraethylammonium hydroxide reagents produced a yellow color with captan. The former spray did not produce a color with captafol and neither spray detected DCNA.

Pomerantz and Ross[68] described a TLC method which differentiates among captan, folpet, captafol and related compounds based on silica gel chromatography with 1% methanol in chloroform as solvent, followed by sequential color develop-

ment of spots with N,N-dimethyl-*p*-phenylenediamine (DMPD), $KMnO_4$, and chromic acid sprays. Table 5 shows these results.

Fishbein *et al.*[73] quantitatively determined captan and captax on silica gel channel chromatoplates by both densitometry at 420 nm and measurement of spot area (width of channel × height of zone). Resorcinol reagent detected captan at R_F 0.35 as a yellow spot with yellow fluorescence under 366 nm UV light (1-μg and 0.5-μg detection limits) after chromatographic development with chloroform. Plots of optical density *vs.* weight and $\sqrt{}$area *vs.* weight were linear between 1–10 μg and 1–20 μg, respectively. Captax was detected by cupric chloride reagent as a yellow spot with R_F 0.25 (2-μg limit) after development with isopropanol–ammonia–chloroform (50:10:40). Densitometry and spot area plots were both linear from 2–16 μg. The feasibility of these methods was demonstrated by the quantitative recovery of 200- and 800-μg amounts of captan from extracts of spiked mouse tissue.

B. Cela W 524

The transport of this systemic fungicide in barley plants was studied by Bruchhausen and Drandarevski[74] using bioautographic TLC against *Cladosporium cucumerinum*. Methanol extracts of plants were developed on silica gel F_{254} layers with water-saturated benzene–ethyl acetate (1:1) followed by diethyl ether in the same direction, thereby separating the fungicide from plant pigment co-extractives. R_F was approximately 0.3 after these two developments, and 0.2 μg of fungicide was detectable.

C. Chloranil

Burke and Holswade[75] reported three peaks for chloranil with retention times relative to aldrin (3.5 min) of 0.37 (major peak), 1.57, and 1.73 on a 6-ft. glass column of 15% QF-1/10% DC-200 (1:1, w/w) on Gas-Chrom Q at 200°C. Five nanograms produced one-half full scale deflection (major peak) with a tritium electron capture detector. The same packing in a 6-ft. aluminum column at 210°C produced one peak with a retention time of 0.44 relative to aldrin (5.5 min) and 8 μg was necessary to produce one-half full scale deflection with a chloride microcoulometric detector. Barrette and Payfer[76] reported chloranil retention times on a 6-ft. 20% DC silicone grease column of 5.2 min at 190°C with 30 p.s.i. (at inlet) helium gas flow and 0.70 min at 230°C with 50 p.s.i. helium.

D. Chloroneb

Formulations of chloroneb were assayed by GLC on a 6-ft. × $\frac{1}{4}$-in. column packed with 20% SE-30, 200°C, with thermal conductivity detection and biphenyl as internal standard. At a flow-rate of 50 ml/min, chloroneb had a retention time of *ca.* 11 min[77]. Residues of chloroneb and its metabolite 2,5-dichloro-4-methoxyphenol were determined utilizing programmed-temperature chloride microcoulometric GLC after separation from the sample by steam distillation. A 4-ft. × $\frac{1}{4}$-in. 10% DC-560 silicone oil plus 0.2% Epon Resin 1001 column programmed from 100° to 180°C and a carrier flow-rate of 50 ml/min separated chloroneb and the metabolite with

respective retention times of 10.7 and 9.3 min. Minimum detectability was 0.01–0.05 ppm for both compounds in a variety of crop, soil, food, and biological samples.

TLC of radio-labeled compounds has been used in various studies of the metabolism of chloroneb. Hock and Sisler[78] used silica gel developed with ethyl acetate–methanol (10:1), while Rhodes and co-workers[79,80] employed silica gel developed in chloroform (R_F chloroneb, 0.6–0.8) for the separation and detection of various metabolites.

E. Chloronitropropane

Yaffe *et al.*[81] determined technical and formulated chloropropane by GLC using 20% DC-550 or DC-710 columns at 100 °C and thermal conductivity detection. Cullen and Stanovick[82] determined chloronitropropane on food crops between 10 and 100 ppb by GLC-ECD with a 4% XE-60 liquid phase at 80°C. The residue was extracted with benzene–methanol (2:1) and the filtered and water-washed extract directly injected into the GLC column. Low chromatograph temperatures allowed interfering plant extractives to be trapped in the injection port (held at 120°C), but a Florisil batch cleanup procedure was used when necessary (*e.g.*, for green vegetables). The injection port required daily cleaning immediately after analysis of all crop extracts. The absolute sensitivity of the system was 25 pg of the fungicide, and the retention time was 3 min.

2-Nitropropane, a metabolite of chloronitropropane, was extracted from cottonseed with ethyl acetate and analyzed by GLC-ECD without cleanup at 0.05- to 0.2-ppm levels[83]. With a 6-ft. 5% Carbowax 20M column at 150°C and a carrier gas flow-rate of 60 ml/min, the metabolite eluted in *ca.* 2.5 min while the parent eluted with the solvent. Calibration curves were linear from 0.02 to 0.6 ng and recoveries were 85 $\pm$ 11%.

F. Chlorothalonil

The method proposed by the Diamond Shamrock Corp.[61] for analysis of this fungicide and its metabolite 4-hydroxy-2,5,6-trichloro-isophthalonitrile in potatoes at 0.01 ppm (ECD) or 0.02 ppm (microcoulometric detector) includes simultaneous extraction with acidified acetone, cleanup and separation of the residues on a partially deactivated Florisil column eluted with 5% and then 50% acetone in dichloromethane, conversion of the hydroxy metabolite to the methyl ether with diazomethane, and GLC analysis of the parent and the derivative. A 6-ft. column packed with 20% DC-200, a temperature of 275°C, and a carrier (helium) flow-rate of 140 ml/min was recommended for microcoulometric detection and a 5-ft. column of 14% Hi-Vac Silicone at 235 °C and 40 ml/min flow-rate of helium for electron capture detection. The retention time was *ca.* 2.5 min for both the parent (5% eluate) and metabolite (50% eluate) in both GLC systems. The Food and Drug Administration[61] tested the procedure with a 10% DC-200 column (200°C) and an ECD and found a retention time of 0.6 for chlorothalonil relative to aldrin, a sensitivity of about 500 pg for one-half full scale deflection, and 93–119% recoveries at 0.1- and 0.2-ppm levels.

G. DCNA

Beckman and Bevenue[84] determined DCNA residues in fruit with a sensitivity of 0.01 ppm using Florisil cleanup and microcoulometric GLC with a 6-ft. × 5-mm column of 20% DC-11 on Chromosorb P at 210°C. With a carrier flow-rate of 100 ml/min, DCNA eluted in *ca.* 4 min. Cheng and Kilgore[85] used a 5% DC-11 column at 185°C and electron capture detection to determine DCNA in benzene extracts of unprocessed stone fruits without cleanup. Brewerton *et al.*[86] determined DCNA down to 0.1 ppm on fruits and vegetables by GLC-ECD with a 5% QF-1 column at 182°C.

The Upjohn Co.[87] recommends a microcoulometric GLC method for determination of DCNA residues (0.1–10 ppm) in fruits, vegetables, soils, and garlic. The residue is extracted with benzene, transferred into acetonitrile, cleaned up by partition with hexane and additionally Florisil (deactivated with 4–10% water) column chromatography (benzene eluent) for products containing chlorophyll, and determined on a 4-ft. column of 5% DC-200 on Anakrom ABS at 150–160°C. The retention of DCNA was 0.47 relative to aldrin and sensitivity was 2 μg at 64 ohms.

Keswani and Weber[88] studied the TLC of DCNA along with seventeen other substituted nitroanilines and related compounds. Silica gel G layers developed with hexane–acetone (3:1) produced an R_F value of 0.42 for DCNA, which was naturally yellow on the plate and remained yellow after diazotization, α-naphthol, or ferric chloride–potassium ferricyanide detection reagents were applied. Von Stryk[89] separated DCNA and its metabolites *p*-nitroaniline, 2-chloro-4-nitroaniline, and 2,6-dichloro-1,4-diaminobenzene by two-dimensional development on silica gel layers with hexane–acetone (3:0.5) followed by benzene–chloroform (8:2). The R_F values of DCNA in these solvents were 0.32 and 0.28, respectively. Detection was made at the 0.5-μg level by exposing the dried plate to 350-nm UV light. The method was tested on spiked plant substrates which were extracted with benzene and cleaned up on a Florisil column.

The GLC-ECD determination of DCNA and its major metabolite 2,6-dichloro-4-hydroxyaniline in tissue and excreta at 0.1 ppm was described by Moseman[90]. After extraction with hexane or acetonitrile and cleanup, if necessary, by partitioning and on Florisil or silica gel columns, DCNA was chromatographed on 3% OV-1 (180–195°C), 5% OV-210 (155°C), or 4% SE-30/6% OV-210 (185°C) columns, depending upon the sample. DCNA and the phenol metabolite were simultaneously determined in urine after acid hydrolysis, neutralization with base, extraction with benzene, and preparation of the chloroacetate derivative of the metabolite, by GLC on an OV-210 column at 160 °C. Aldrin eluted in *ca.* 4 min, DCNA in 5 min, and the dichloroaminophenol in 6 min with a nitrogen flow-rate of 70 ml/min.

Van Alfen and Kosuge[91] employed preparative silica gel TLC to isolate DCNA metabolites from culture fluids in their study of the microbial metabolism of this fungicide. Metabolites were separated and detected by analytical thin-layer radiochromatography with benzene–diethyl ether (1:1) and chloroform–acetone (7:3) mobile phases.

H. Dichlofluanid

Strawberries treated with this fungicide during growth were analyzed for the

parent and breakdown product dimethylaminosulfanilide by GLC-ECD[92]. Two different 5-ft. × 1/8-in. columns containing 5% DC-11 and 5% QF-1 at 180°C gave retention times of 4.5 and 3.0 min, respectively, with a nitrogen flow-rate of 50 ml/min. The sulfanilide had a retention of 1.5 min on the latter column and was not detected on the former. Residues were extracted by shaking the frozen or canned sample with benzene, the aqueous phase was discarded, and the benzene layer dried over sodium sulfate, filtered, concentrated if necessary, and chromatographed without cleanup. Dichlofluanid was detected at 0.01- to 1.6-ppm levels, while no residues of the sulfanilide were found in any sample tested.

I. Dichlone

Benzene was used to extract dichlone from fruit samples, the extracts were dried over sodium sulfate, and cleanup carried out on a Florisil column, if required[93]. GLC-ECD on a 5-ft. column of 5% QF-1 provided linear calibration curves over a range equivalent to 0.3–5.0 ng dichlone, which eluted in just under 6 min. Samples should be analyzed quickly after extraction to preclude degradation and conversion of the fungicide. Recoveries were poor at lower fortification levels (50% at 0.01 ppm, 90% at 1.0 ppm) and when the length of time between extraction and analysis increased[94].

The fungicides dichlone, Bulbosan, fuberidazole, and chlorothalonil were detected following cellulose and silica gel TLC by spraying the plate with a reagent which forms a π-complex with the pesticides. Various reagents (TNF, CNTNF, TCNE, etc.) were tested with pesticides at 5 μg/spot levels and characteristic colors resulting were tabulated. Neither detection limits nor practical analytical applications were included in this report[95]. A TLC method for 0.01–0.05 ppm dichlone in grape leaves and berries or apples involved extraction, purification by microsublimation, and development on silica gel G using cyclohexane–chloroform (7:3)[96].

J. Dichlozoline

Pack *et al.*[97] analyzed grapes and grape products by extraction with hexane, cleanup of low levels (< 0.5 ppm) of residues in certain samples on a silica gel column eluted with hexane–benzene (1:1), and GLC-ECD on a 5-ft., 2% DEGS (185°C) or 2-ft., 5% QF-1 (150°C) column. With a carrier flow-rate of 30 ml/min, retention times were *ca.* 4 min and 7 min, respectively, and 1 ng gave about one-half full scale recorder deflection. Detection limits were 0.01 ppm and recoveries at 0.01–1 ppm were excellent. A similar GLC-ECD method for analysis of the same products was reported by Mestres *et al.*[98]. The sample was extracted with light petroleum in the presence of sodium sulfate and Celite to reduce emulsions, and the extract was then analyzed by GLC at 0.01- to 0.5-ppm levels. At levels below 0.25 ppm, Florisil column cleanup was required.

K. Drazoxolone

Yuen[99] recommended colorimetry at 400 nm for the analysis of both formulations and residues of drazoxolone. Extracts of certain plant species such as grass

contain pigments which caused high background absorption readings. Chromatographic cleanup through a 10-g column of Florisil was carried out, drazoxolone being selectively eluted with chloroform.

L. Dyrene

Burke and Holswade[75] reported a retention time for this triazine fungicide of 2.29 relative to aldrin on a 15% QF-1/10% DC-200 (1:1) column at 200°C with electron capture detection. 40–50 ng Dyrene caused one-half full scale deflection. Using the same column at 210 °C but with a microcoulometric detector, the same workers reported five peaks for Dyrene with relative retentions of 1.33 (major), 1.77, 2.08, 2.37, and 2.67 and a sensitivity of 25 μg.

Wales and Mendoza[100] obtained the recovery of 5–20 ppm Dyrene from plant samples by acetonitrile extraction and hexane partitioning with analysis by GLC-ECD on SE-30/QF-1 or OV-17/SE-30/QF-1 mixed phases[101] at about 210°C. The sensitivity of the detector was $\frac{1}{2}$ f.s.d. to 5 ng Dyrene. Confirmation was obtained by reaction of the extracts with methanolic NaOH prior to GLC to produce two major products with longer retention times than Dyrene. Captan, if present, would also be recovered by this procedure and would be separated from Dyrene on the SE-30/QF-1 column. The methanolic NaOH treatment would destroy captan.

M. HCB

Besides those mentioned at the start of this article, several other multiresidue studies have included the fungicide HCB. Recovery of 92% at the 0.025-ppm level was obtained from dry poultry food and grain by the TLC cleanup method of Heatherington and Parouchais[102] prior to GLC. Development of samples on aluminum oxide G layers was with acetonitrile–tetrahydrofuran (1:1) in an unsaturated tank for 10 cm followed by air drying and re-development with acetonitrile for 12 cm. The alkaline precolumn procedure of Miller and Wells[103] was used to destroy certain pesticides prior to chromatography on a separate analytical GLC column, while other pesticides were converted to chromatographable derivatives with altered retention times and still others, including HCB and TCNB, were unchanged. This procedure eliminated many background peaks and offered evidence useful in confirming residue identity. HCB had a retention of 0.49 relative to aldrin on a 10% DC-200 column at 210°C[104], and 0.15, 0.25, and 0.12 relative to dieldrin on 1.3% columns of SE-52 (160°C), Apiezon L (190°C), and XE-60 (200°C), respectively, each containing 0.1–0.2% Epikote resin 1001 (ref. 105). The chromatography of HCB on a 5% QF-1 column is illustrated in Fig. 1.

The TLC of HCB has been reported[106] in the following systems: alumina layers/*n*-heptane solvent ($R_{DDD} = 2.7$), alumina/2% acetone in heptane (1.7), alumina impregnated with 25% N,N-dimethylformamide (DMF)/isooctane (5.7), and silica gel G-HR/1% acetone in heptane (2.5).

Problems encountered in the analysis of HCB residues in the ppb range in cereals were studied by Taylor and Keenan[107]. Extraction by refluxing with hexane, separation from interfering grain lipids by steam distillation, separation from α-BHC insecticide on a 5% Reoplex GLC column, and an alkaline degradation confirmatory

procedure were offered as partial solutions to some of the difficulties. α-BHC and HCB were not separated on some of the usual pesticide GLC columns but had $R_{dieldrin}$ values of 12 and 29, respectively, on a 5-ft. Reoplex column at 200°C.

Smyth[108] detected HCB in dairy products, meat fat, and eggs with a lower detection limit of 0.002 ppm (1-g fat sample) and recoveries in excess of 80%. After hexane extraction of fat, cleanup was on a deactivated Florisil column eluted with dichloromethane–hexane (2:8) and GLC-ECD on a 1% DC-200/1% QF-1 column supported on Varaport 30. At 185°C, HCB had an R_{aldrin} value of 0.40 (0.47-min elution time on a 6-ft. × 2-mm column, 18 ml/min nitrogen flow-rate) and was separated from α-BHC and other common chlorinated insecticides. Florisil deactivation at a level (*ca.* 0.5–1.5% water) which recovered 80% of added dieldrin provided adequate cleanup of 1-g fat samples.

DiMuccio *et al.*[109] designated GLC phases for the separation of HCB from BHC isomers and other chlorinated pesticides. Table 6 shows retention times on the recommended columns.

Weber *et al.*[110] used phenyl ether derivative formation (phenol/KOH reaction) to eliminate interference of contaminants in analyses of meat brei for HCB. Retentions relative to lindane on XE-60, SE-30, and DC-200 GLC columns were 2.2, 5.5, and 5.1 min, respectively, for the ether derivative.

Curley *et al.*[111] screened adipose tissue from people of Japan and detected < 0.003–0.77 ppm HCB among other chlorinated pesticides in 241 samples. GLC-ECD on columns of 1.5% OV-17/1.95% QF-1 or 5% OV-210 were used to separate and detect low picogram quantities after extraction and cleanup by the traditional Mills procedure, which is known to provide low recovery of HCB. Confirmation of this fungicide was made by Coulson conductivity detection, TLC, and combined

TABLE 6

RETENTION TIMES RELATIVE TO ALDRIN OF CHLORINATED PESTICIDES[109]

Compound	*DC-200/QF-1/XE-60**	*OV-61/QF-1/XE-60***
HCB	0.41	0.40
α-BHC	0.53	0.54
γ-BHC	0.70	0.73
Heptachlor	0.83	0.82
Aldrin	1.00 (8.9 min)***	1.00 (7.9 min)§
β-BHC	1.20	1.15
δ-BHC	1.29	1.31
Heptachlor Epoxide	1.60	1.74
p,p'-DDE	2.16	2.42
Dieldrin	2.46	2.74
o,p'-DDT	2.65	3.39
p,p'-DDT	3.78	4.36

* 1:1:1 mixture of three previously coated packings: 10% DC-200 on HP Chromosorb W, 7.5% QF-1 on Chromosorb W HP, and 3% XE-60 on silanized Anakrom AS; all percentages by weight, supports 80–100 mesh.

** 1:1:0.5 mixture of 3% OV-61 on 80–100 mesh silanized Gas-Chrom P plus QF-1 and XE-60 as above.

*** 2-m × 4-mm column, 190 °C, argon–10% methane carrier gas, 40 ml/min, ECD in pulsed mode.

§ As above, but 2-m × 3-mm column.

GLC–mass spectroscopy. The mass spectrum indicated all fragments caused by successive loss of chlorine from the parent ($C_6Cl_6^+$) to $C_6Cl_1^+$. TLC on 2-mm preparative silica gel fluorescent layers with light petroleum–diethyl ether–acetic acid (90:10:1) provided a quenched HCB spot with an R_F value of about 0.66 under UV light.

Wollenberg and Drossel[112] determined residues of HCB in meat products by its conversion into a mixture of ether derivatives by treatment under mild conditions (60°C) with potassium phenoxide solution in dimethylsulfoxide. The derivatives were separated by GLC, and the peak from the chief product [pentachloro(phenoxy)-benzene] was used to provide quantitation of HCB content.

Confusion between elemental sulfur and HCB on thin-layer chromatograms may be decreased by UV irradiation of the layer prior to application of the $AgNO_3$ detection spray[113].

Holdrinet[114] determined and confirmed HCB in fatty samples at low ppm levels in the presence of other residual haloginated pesticides and PCBs. Following initial hexane extraction and Florisil cleanup, organochlorine pesticides, PCBs, and HCB were successively eluted from a Fisher 5-690 charcoal column with acetone–diethyl ether (1:3), benzene, and toluene. HCB in the third fraction was determinated by GLC-ECD with a 4% SE-30/6% QF-1 column at 180°C. The HCB fraction was then subjected to caustic alkali at high temperature and the hydrolyzed product methylated to yield pentachlorophenol methyl ether derivative for confirmation.

N. Hexachlorophene

Although gas chromatography has been carried out directly[115,116] on hexachlorophene, most residue analyses have involved formation of methyl derivatives. The GLC-ECD analysis of hexachlorophene in cucumbers, tomatoes, corn, and milk at 0.02- to 0.2-ppm levels was reported by Gutenmann and Lisk[117]. After extraction with acetone, filtration, and cleanup by chloroform partitioning and batch treatment with Celite–H_2SO_4, the compound was methylated and chromatographed on a 2-ft. × 6-mm 10% DC-200 column at 200°C. One nanogram of methylated fungicide gave a peak with one-half full scale recorder response and a retention time of 16.5 min (75 ml/min carrier flow-rate). Ferry and Queen[118] also used GLC-ECD for the analysis of hexachlorophene in blood. Extraction was performed with diethyl ether, followed by methylation, addition of hexane to dissolve the methyl ether, and cleanup of the hexane phase by extraction with H_2SO_4 and saturated sodium sulfate. Concentrates were chromatographed on a 0.5-m × 2.5-mm 5% QF-1 column at 185°C with a carrier flow-rate of 40 ml/min. Retention of the methyl ether was 4.5 min, and linear GLC response was obtained over the range 0.005–0.200 ng.

Formation of trimethylsilyl (TMS) derivatives[119] and acetylation[120] have also been suggested for GLC analysis of hexachlorophene. This latter approach was chosen by Greenwood *et al.*[121] for determination of traces of the fungicide in blood. Whole blood (3 ml) was partitioned with ethyl acetate (10 ml), the extract concentrated, reacted with a mixture of equal volumes of acetic anhydride and pyridine (0.1 ml), and determination made by GLC with a ^{63}Ni ECD. A 3% OV-17 column at 265°C was employed and detection was made at a blood level of 330 pg of hexachlorophene per ml.

French *et al.*[122] studied variations in separability and sequence by TLC on different silica gel precoated plates for the mixture hexachlorophene, trichlorocarbanilide, and tribromosalicylanilide. With benzene–diethyl ether (8:2) as solvent, R_F values of hexachlorophene ranged from 0.07–0.36, while the range was 0.02–0.14 with hexane–ethyl acetate (7:3). The elution order for the three compounds was altered on different brands of layers, emphasizing the importance of using chromatographic conditions as similar as possible if published work is to be reproduced.

Carr[123] analyzed hexachlorophene by HSLC with a 50-cm × 0.2-cm MicroPak SI-10 small-particle (< 10 μm), porous silica gel column connected in series with a variable wavelength UV detector set at 296.5 nm. Hexachlorophene was eluted in *ca.* 1 min with hexane–dichloromethane–isopropanol–glacial acetic acid (89:8:1:2), obtaining a minimum detectable quantity of 10 ng. Using this optimum wavelength, a similar sensitivity as reported by Porcaro and Subiak[124] was achieved without formation of the higher absorbing di-*p*-methoxybenzoate ester and the resulting greater sample manipulation and analysis time required with the fixed-wavelength 254-nm detector used by the latter workers.

O. Parinol

Day *et al.*[125] described methods for determination of parinol in formulations, soils, and plant tissues. A GLC column of 1.5% OV-17 at 230°C was found to be generally applicable. An FID was suitable for formulation analysis and an ECD for trace analysis. Parinol eluted from a 4-ft. × 3-mm column in *ca.* 6 min with a flow-rate of 40 ml/min. Residues as low as 0.01–0.02 ppm in various crop and soil samples were determined by blending the sample with acetone, partition into hexane, extraction of the hexane solution with 0.5 *N* HCl, addition of base, and extraction with chloroform. The chloroform solution was evaporated to dryness and dissolved in a small volume of benzene for GLC as above.

If residues were present at levels greater than 0.1 ppm, a TLC procedure was successfully employed. The benzene solution of the chloroform extract was spotted on a silica gel layer and developed in diethyl ether–hexane–methanol (80:18:2) in an unsaturated chamber. The developed plate was sprayed with 10% H_2SO_4 in diethyl ether and heated for 5 min at 110°C. A red parinol spot appeared which became yellow on exposure to cool air. The limit of detection was about 0.2 μg. Quantitation was carried out by visual comparison of samples and standards on the same plate, or by removal of the spot from the plate, elution from the silica gel, evaporation of the solvent, development of the red color in H_2SO_4, and colorimetric analysis.

P. PCNB and TCNB

Klein and Gajan[126] determined PCNB and TCNB residues on lettuce, cabbage, and string beans by chloride microcoulometric GLC at 0.1–5 ppm using a 20% DC silicone grease column at 220 °C. Both PCNB and TCNB were separated and measured individually. Burke and Holswade[104] reported a retention time of 0.54 min relative to aldrin for PCNB and 0.36 for TCNB on a column of 10% DC-200 at 210°C with a microcoulometric detector. Sensitivities were 0.75 and 1 μg, respectively. Gorbach and Wagner[127] analyzed PCNB in potatoes by microcoulometric GLC on a silicone grease column at 170 °C and identified one of two metabolites as

pentachloroanaline. Methratta *et al.*[128] determined PCNB in vegetables, fruits, seeds, and soil at 0.01–0.3 ppm by GLC-ECD on a 4-ft. × ¼-in. 2% SE-30 column at 170°C. Samples were extracted with hexane and interferences removed on a silicic acid (10% moisture) column eluted with hexane. PCNB eluted in about 94 sec with a nitrogen flow-rate of 160 ml/min and the sensitivity was 150 pg for full scale response with a 0.3% noise level. Caseley[129] determined PCNB and TCNB residues in soils, after mechanical extraction with acetone and partition with hexane, by GLC-ECD on a 5-ft. × 1/8-in. column of 5% SE-30 at 175°C. Retention times were 3.0 and 1.5 min, respectively, at a nitrogen carrier flow-rate of 70 ml/min.

The GLC procedure of Methratta *et al.* (above) was adopted by Kuchar *et al.*[130] to study the metabolism of PCNB in beagle dogs, rats, and plants. Extraction was made with acetonitrile or hexane, depending on the sample. The metabolic products of PCNB were found to be pentachloroaniline (retention 1.4 relative to PCNB on 2% SE-30) and methyl pentachlorophenyl sulfide (2.0) in all three instances.

Photoreduction of the fungicides PCNB, PCP, and PCB by 254 nm UV light and sunlight was studied[131] using programmed-temperature thermal conductivity or chloride microcoulometric GLC with 20% DC-11 or 10% SE-30 columns. Preparative GLC was accomplished with an FID chromatograph and 5% DC-11 column. Programming between 110–220 °C provided separation (in order) of 1,2,4,5-tetrachlorobenzene (first eluted), PCB, 2,3,4,6-tetrachloronitrobenzene, 2,3,4,5-tetrachloronitrobenzene, and PCNB.

Residues (0.01–5 ppm) of PCNB in tomatoes, lettuce, and bananas were determined by Baker and Flaherty[132]. After extraction with hexane, PCNB was separated from interfering co-extractives by partition with DMF followed by chromatography on a deactivated alumina (5% water) column eluted with hexane and was quantitatively determined by GLC-ECD on a 5% EGSS-X column at 170 or 200 °C (retention PCNB = 0.19 or 0.25, relative to dieldrin). The limit of detection was 5 pg at a signal-to-noise ratio of 3:1, and response was linear from 0.1–1.0 ng. GC columns containing 1.3% SE-52/0.15% Epikote 1001 at 200 °C (relative retention = 0.25) and 1% Apiezon L/0.15% Epikote 1001 at 196 °C (0.26) were suitable for confirmation. A chemical confirmatory test for PCNB was also described, namely reduction with lithium aluminium hydride in diethyl ether to form pentachloroaniline (PCA), the formation of which was confirmed by GLC before and after shaking the reduced extract with H_2SO_4 to remove PCA. Any PCA present in untreated samples as a natural metabolite of PCNB was removed by treatment with H_2SO_4 prior to reduction. Lettuce samples known to have been treated with thiram and zineb gave GLC peaks which eluted in the vicinity of PCA and interfered with the PCA determination. These peaks, which were attributed to sulfur-containing degradation products of the fungicides, were removed from the organic extract by elution through a silver nitrate–alumina column prior to carrying out the confirmatory test.

Griffith and Blanke[133] included PCNB, captan, and folpet among 31 compounds in their improved microcoulometric method for organochlorines in blood. Results are shown in Table 7.

Q. PCP (see also Section 7H)

A rapid method[134] for determination of PCP in 2 ml human blood serum was

TABLE 7

MICROCOULOMETRY OF FUNGICIDES IN BLOOD[133]

Compound	*RRT**		*Response***		*Recovery****	
	A§	*B*§§	*ECD*§§§	*MC*†	*ng*	*%*
PCNB	0.70	0.91	1.5	2, 1	60	91
Folpet	1.85	2.53	20	20, 40	200	55
Captan	1.88	2.59	20	20, 40	200	55

* Retention time relative to aldrin, which has a retention of 3.6 min in system A and 4.7 min in B.

** Ng for one-half full scale deflection.

*** Recovery of fungicides added to 2 ml of whole blood at the middle level reported using a modified H_2SO_4 extraction method.

§ Column A: 6-ft. × 4-mm glass column packed with 4% SE-30/6% QF-1 on 80–100 mesh Supelcoport, 205 °C, nitrogen carrier gas flow-rate 120 ml/min.

§§ Column B: 6-ft. × 4-mm glass column packed with 5% OV-210 on 80–100 mesh Gas-Chrom Q, programmed at 2°C/min from 210–234 °C, initial hold 1 min, final hold 4 min, nitrogen carrier gas flow-rate 90 ml/min.

§§§ Column A.

† Response for chloride microcoulometric detector on columns A, B.

based on its conversion to a methyl ether with diazomethane after a 2-h extraction of the acidified sample with benzene. GLC-ECD on 4% SE-30/6% QF-1 or 5% OV-210 columns at 200°C was utilized for quantitation, comparing sample peaks against peaks from standards similarly methylated. Because of the widespread prevalence of PCP, a reagent blank must be carried through the entire procedure along with the samples. The method had a lower detection limit of 10 ppb with a detector sensitivity of 50 pg or less PCP methyl ester. A similar method for analysis of blood plasma or urine was reported by Rivers[135].

For determination of PCP residues in 5 ml human urine[136], the sample was made alkaline and extracted with hexane to remove basic and neutral interferences. The alkaline urine was acidified and re-extracted, PCP derivatized with diazomethane, and the alkylated PCP determined by GLC-ECD. Confirmation was achieved by *p*-value[137] determinations, GLC retention times on several columns, or preparation of additional alkyl ether derivatives of PCP[138]. Retention times of methylated PCP relative to aldrin on several recommended columns were as follows: 1.5% OV-17/1.95% QF-1 (200 °C), 0.47; 4% SE-30/6% QF-1 (200 °C), 0.63; and 5% OV-210 (180 °C), 0.56. Forty picograms of PCP ether resulted in a quantifiable peak, this amount corresponding to a level of 2 ppb PCP in the original urine sample.

A method for the analysis of PCP residues on nuts and stone fruits also employing methylation and electron capture detection was reported by Kilgore and Cheng[139]. Acidified samples were extracted with benzene, a sulfuric acid washing procedure was used for cleanup when required, and analysis was on a 5% DC-11 column at 180°C. Sensitivity was reported as 0.01 ppm. Bevenue *et al.*[140] used a similar method for determination of PCP in urine. Columns of 5% QF-1 and 10% DC-200 at 145°C and an ECD provided a linear range of 30–400 pg PCP. Other early analytical methods for PCP were reviewed by Bevenue and Beckman[141]. Bevenue *et al.*[142] later reported another similar method for PCP in human blood. Simultaneous

application of acid pH, mild heat, and agitation of the sample with benzene isolated the fungicide from 1–5 ml of sample, followed by methylation with diazomethane and GLC–ECD on 5% QF-1 and 3.3% DC-200 columns. Detectability limits were in the low ppb range.

Barthel *et al.*[143] determined PCP in blood, urine, tissue, and clothing as part of an investigation of illness and fatalities in a nursery treated with a mildew preventive containing PCP. Samples were extracted with diethyl ether, the ether solution extracted with 5% NaOH, the basic solution acidified and extracted with benzene, and this solution analyzed by GLC on a 3% DEGS column containing 2% syrupy H_3PO_4. This column at 150 °C with a ^{63}Ni ECD at 280°C allowed detection of PCP at the 0.02-ng level without derivatization. H_3PO_4 reduced the polarity of PCP and elution from the 4-ft. column was obtained in *ca.* 2 min.

Stark[144] used GLC-ECD for determinations of PCP in soil, water, and fish at 0.05- to 2-ppb levels after extraction, partition, and methylation. TMS derivatives were prepared for GLC confirmation. Higginbotham *et al.*[215] analyzed fats, oils, and fatty acids for PCP and 2,3,4,6-tetrachlorophenol by GLC-ECD on a 7-ft. 10% DEGS/2% H_3PO_4 column at 170°C. PCP eluted in *ca.* 25 min and the phenol in 10 min. Extraction of acidified samples was made with light petroleum followed by partition with aqueous alkali and chloroform to separate the phenols. After further treatment with H_2SO_4, GLC was carried out. Recoveries were reportedly low and variable and detection only at the 0.5-ppm level was claimed. TLC on Gelman ITLC Type SG sheets with light petroleum–heptane (1:1) solvent and detection under 350-nm UV light after spraying with Rhodamine B reagent yielded orange-pink spots (0.5–5 μg) at R_F 0.4–0.5 for both phenols in addition to 2,4,5-trichlorophenol. Phenols were further studied as impurities in PCP formulations by GLC-mass spectrometry on a temperature-programmed 4% SE-30 column after methylation[145].

The acetate derivative of PCP was recommended by Chau and Coburn[146] for the determination of PCP in natural and waste water at levels as low as 0.01 ppb/l. PCP was extracted with benzene and from the benzene into potassium carbonate solution. The addition of acetic anhydride to the aqueous alkali extract produced the acetate of PCP, which was extracted by hexane and analyzed by GLC-ECD on OV-17/QF-1 or OV-101/OV-210 GLC columns. Advantages of the acetyl derivative over the usual methyl ether derivative were found to be simplification of the procedure because of derivative formation in aqueous solution and a larger linear range of ECD (^{63}Ni) response. Seventeen other phenols were found not to interfere in the analytical procedure.

PCP was detected on thin-layer chromatograms along with other chlorinated phenols after fluorigenic labeling with dansyl chloride[147]. The dansyl derivative of PCP had an R_F value of 0.83 on a silica gel layer developed with benzene–chloroform (1:1). The nature of the derivative was investigated by scanning fluorescence *in situ* with a Zeiss PMQ II chromatogram spectrometer. Neither the sensitivity of the method nor calibration curves were presented, although it seems likely that densitometric quantitation of PCP residues in the low nanogram range could be made using this technique.

The cleanup of the fungicides PCP, 2,4,6-trichlorophenol, and 2,3,4,6-tetrachlorophenol and phenoxy acid herbicides by batch and column ion-exchange procedures was described by Renberg[148]. The acidic residues were bound under alkaline

conditions to a strong base anion-exchange resin and removed subsequently under acid conditions. Gas chromatography of methylated derivatives of the fungicides was carried out, after ion-exchange cleanup, on columns of 1% OV-17 (160°C) and 8% QF-1/4% SF-96 (150°C). PCP was determined at levels as low as 0.35 ppm in contaminated water and fish tissue.

Colvin *et al.*[149] added PCP as the internal standard for the formulation analysis of carbaryl insecticide by high-pressure liquid chromatography. With a 2-ft. × 1/8-in. Carbowax/Porasil column and 20% chloroform in isooctane as solvent at a flow-rate of 1.5 ml/min, 4 μg PCP was eluted in 1.5 min and gave 60% f.s.d. with a UV photometric detector.

R. Triarimol

Frank *et al.*[150] analyzed formulations of this fungicide by GLC-FID after the sample had been dissolved in or extracted with chloroform. Triarimol eluted in *ca.* 4 min from a 4-ft. × 3-mm column of 2% OV-17 with a carrier gas flow-rate of 35 ml/min. Dibenzyl phthalate was a suitable internal standard. GLC columns packed with UC-W98 and JXR liquid phases were also successfully used. Pesticides commonly occurring with triarimol, including the fungicides captan and maneb, did not interfere with the analysis.

7. MISCELLANEOUS FUNGICIDES

A. BAS-3191

The metabolism of this new systemic anilide fungicide was studied employing silica gel TLC and alumina column chromatography to purify and separate the parent compound and its metabolites prior to determination and identification by spectroscopy. Benzene–acetic acid (9:1) and chloroform–acetone–acetic acid (15:2:3) were solvents for TLC, and chloroform was used to fractionate compounds on the column[151].

B. Benomyl

The standard analytical method for benomyl residues involves determination at 0.1 ppm (25-g sample) by either direct fluorimetric measurement or by colorimetric analysis following bromination, after conversion to 2-aminobenzimidazole. Benomyl is quantitatively converted to methyl 2-benzimidazolecarbamate (MBC) and then to 2-aminobenzimidazole (2-AB) by a two-stage acid–base hydrolysis procedure after extraction from the sample substrate with ethyl acetate. The extract is purified by liquid–liquid partitioning steps. In addition to this procedure[152], which measures benomyl, its principal degradation product MBC, and a minor component in plants, 2-AB, as a composite value, others involving chromatography have been developed as outlined below.

Rouchaud and Decallonne[153] determined benomyl and MBC hydrolysis prod-

uct in plants and soil by extracting residues with benzene, partitioning into 0.1 *N* HCl, washing the acidic layer with chloroform and then neutralizing. At this point the residues were completely in the form of MBC (present initially or formed during acid hydrolysis), which was partitioned into ethyl acetate, trifluoroacetylated, and measured by GLC-ECD on a 1.5-m × 2.2-mm 5% SE-30 column at 140°C. The MBC-TFA derivative had a retention time of 3.1 min, with a carrier flow-rate of 40 ml/min. Parathion, used as an internal standard, eluted in 7.9 min. The limit of sensitivity was 0.02 ppm.

Technical samples of benomyl were separated into two toxic components on silica gel layers developed with ethyl acetate[154]. The breakdown product was identified as MBC. Peterson and Edgington[155] used bioautographic TLC to estimate quantitatively benomyl and MBC. A silica gel Chromagram sheet was sprayed with a mixture of agar and *Penicillium* spores, and the diameter of the zones of inhibited growth on the layer was related to the amount of fungitoxic chemicals present. Development with acetone separated benomyl ($R_F \approx 0.3$) and MBC (≈ 0.5). Amounts equivalent to as little as 0.03 μg benomyl were spotted and two spots were readily detected. The diameter of both spots increased linearly with log concentration (0.03–0.4 μg benomyl equivalent) for both spots. Homans and Fuchs[156] likewise used bioautography on thin-layer chromatograms to detect 1-μg amounts of benomyl after development with diethyl ether or ethyl acetate and location of the pesticide by its UV absorption on Merck silica gel F_{254} aluminum-backed layers. The spray solution was a fungus organism such as *Cladosporium cucumerinum* in a suitable medium.

Mallet *et al.*[157] studied the fluorescence of benomyl and the fumigants fuberidazole and Quinomethionate on silica gel thin layers. Fluorescence spectra were determined *in situ* and the effects of heating the chromatogram at 200°C for 45 min were noted. Heat treatment shifted the emission and excitation maxima and in some cases increased the number of peaks. These changes are useful evidence along with R_F values in confirming residue identity. Limits of detection were also determined after development of layers with hexane–acetone (9:1). Heating may increase or decrease fluorescence intensity. Results are shown in Table 8.

Vogel *et al.*[158] extracted benomyl residues from disintegrated fruits and vegetables with ethyl acetate, hydrolyzed benomyl and/or MBC to 2-AB, and chromatographed this on silica gel layers with detection by exposure to bromine vapor. Yellow-brown spots with an R_F value of 0.4 (hexane–ethyl acetate–methanol, 1:1:1)

TABLE 8

FLUORESCENCE SPECTRAL DATA AND VISUAL LIMITS OF DETECTION ON SILICA GEL THIN LAYERS[157]

Fungicide	*Wavelength**				*Limit of detection (μg)*	
	Natural		*After heat treatment*		*No heat*	*Heat*
	EX	*EM*	*EX*	*EM*		
Benomyl	298	422	362	464	0.06	0.02
Fuberidazole	328	402	323, 373	447	1.00	0.005
Quinomethionate	363	418	335, 360	465, 478	0.004	0.04

* EX = excitation, EM = emission.

were formed with a detectability of 0.2 ppm. Tjan and Burgers[159] determined benomyl and thiabendazole in fruits by ethyl acetate extraction, cleanup by partition with 0.1 *N* HCl, neutralization and extraction back into ethyl acetate, and TLC of concentrates on silica gel G or GF_{254} plates with chloroform–acetone (8:2) solvent. The separated fungicides [R_F 0.35 for thiabendazole, 0.18 and 0.70 for benomyl (two spots) on silica gel GF] were detected as pink-blue spots under 254-nm UV light on fluorescent layers, or more specifically by an enzyme-inhibition method (honey bee extract/2-naphthyl acetate–Fast Blue B) using 0.50-mm non-fluorescent layers. Recoveries at 1–2 ppm levels from a variety of fruit were 75–83% for thiabendazole but only 40–43% for benomyl, probably because of loss of hydrolysis products of the latter through the procedure.

White and Kilgore[160] used a TLC-UV spectrophotometric method to assay various food crops for benomyl and MBC at 0.05-ppm levels. The compounds were extracted with benzene, partitioned into 0.1 *N* HCl, the acidic layer was washed with chloroform, neutralized to pH 8 with NaOH, and the single product MBC present at this point was partitioned into ethyl acetate, concentrated, and chromatographed. Plastic sheets precoated with polyamide II containing fluorescent indicator were prewashed with chloroform–ethyl acetate–acetic acid (190:10:4) solvent, dried, spotted, and developed with the same solvent. MBC spots (R_F 0.7) were detected under shortwave UV light, excised from the plate with a Brinkmann vacuum spot collector, and the compound was eluted with methanol prior to spectrophotometric analysis at 287 nm. White *et al.*[161] used this analytical procedure plus TLC on silica gel F_{254} precoated glass plates with benzene–methanol (9:1) as developing solvent to identify thermal and base-catalyzed hydrolysis products of benomyl. Benomyl standard exhibited an R_F value of 0.48 when detected under 254-nm UV light. Baude *et al.* separated radiolabeled benomyl and plant[162] and soil[163] metabolites by silica gel TLC and quantitated by radioscanning or radioautography and liquid scintillation counting. Solvents employed were ethyl acetate–methanol–NH_4OH (100:25:0.5–1) or ethyl acetate–dioxane–methanol–NH_4OH (320:40:10:1). This method involved the conversion of benomyl to the stable derivative 3-butylureidobenzimidazole while MBC hydrolyzed to 2-AB.

Kirkland[164] used HSLC to determine residues of benomyl and/or MBC, and the hydroxylated metabolites methyl 5-hydroxy-2-benzimidazolecarbamate (5-HBC, a major metabolite in animal urine which was originally identified by TLC[165]) and methyl 4-hydroxy-2-benzimidazolecarbamate (4-HBC, a minor metabolite in animal urine) in cow milk, tissues, urine, and feces. The sample was hydrolyzed in acid to convert benomyl to MBC and to free the metabolites from conjugates. The freed materials were extracted into ethyl acetate, the extract was cleaned-up by solvent partitioning, and determined on a 100-cm × 2.1-mm column of Zipax SCX strong-acid cation-exchange packing with a carrier phase of 0.15 *N* sodium acetate–0.15 *N* acetic acid (7:3) at 60°C. With a carrier flow-rate of 0.5 ml/min, 4-HBC eluted in 7.2 min and 5-HBC in 8.6 min. Fifteen minutes after sample injection the flow-rate was increased to 1.5 ml/min, and MBC eluted 22 min from the start. Recoveries were demonstrated at 0.01- to 0.2-ppm levels (25-g samples), and 28 other pesticides with tolerances in milk and meat caused no interference with this procedure. These included the fungicides thiabendazole and chloroneb. The fate of benomyl was studied in other animals[166] and 5-HBC was indicated as the major metabolite in feces

and urine. TLC on silica gel layers developed twice with ethyl acetate–methanol–glacial acetic acid (100:100:4) followed by methanol–water (2:1) (same direction) or once with ethyl acetate–dioxane–methanol–NH_4OH (160:20:5:0.5) was used for separation of metabolites.

Another method employing Zipax SCX cation exchanger after extraction and liquid–liquid partitioning cleanup was reported by Kirkland *et al.*[167] for benomyl residues (sensitivity, 0.05 ppm) in soils and plant tissues. With a 254-nm UV detector and elution at 60°C and 300 p.s.i.g. using 0.025 *N* tetramethylammonium nitrate–0.025 *N* HNO_3 solvent, peaks with retention times of *ca.* 18 and 22 min for MBC and 2-AB, respectively, were detected.

Another fluorimetric procedure[168] for benomyl, MBC, and the fungicide thiabendazole included cleanup on a magnesium oxide–Celite–alumina column. Thiabendazole was eluted with ethyl acetate followed by benomyl/MBC with ethanol–ethyl acetate (1:1).

C. Binapacryl

Baker and Hoodless[169] determined residues of binapacryl (0.3–0.5 ppm) in selected fruits by extraction with hexane–diethyl ether–DMF (4:1:2), cleanup on an activated silica gel column eluted with hexane–diethyl ether (4:1), and GLC-ECD determination on 3% XE-60 (200°C) or 15% DC-200 (204°C) columns. A confirmatory chemical test was described in which binapacryl was hydrolyzed with methanolic KOH to give the free phenol (DNBP, dinoseb), and the phenol was then methylated with diazomethane to give the corresponding ether which was detected by GLC. The ether had a retention time of 0.29 relative to binapacryl on the XE-60 column. Dinoseb herbicide, a potential breakdown product of binapacryl on the fruit, would not be detected under the GLC conditions used. Dinoseb acetate or dinobuton fungicide, if present, would invalidate the confirmatory test since both of these would form the same ether [2-(1-methyl-*n*-propyl)-4,6-dinitroanisole].

Buxton and Mohr[170] mentioned the GLC-ECD determination of binapacryl residues on cottonseed after extracts were purified by partitioning from hexane into acetonitrile, but no details were given.

D. Biphenyl (see also Section 7L)

Morries[171] determined biphenyl and *o*-phenylphenol in the peel of citrus fruits by GLC-FID following homogenization with diethyl ether and filtration of the extract. A polar column consisting of 15% ethanediol adipate polyester on Chromosorb and fluorene as internal standard were employed. Detection at 1 ppm and 5 ppm, respectively, was made without significant interferences. A GLC method reported by Beernaert[172] employed steam distillation to free biphenyl and *o*-phenylphenol from citrus fruit, extraction of the distillated phase with light petroleum, and GC on a 1% SE-30 column with a temperature program from 50 to 300°C. Benzophenone was used as internal standard for recovery studies at 40 and 20 ppm, respectively. The FID was again employed successfully due to the high levels studied as dictated by the high tolerance levels of residues established in various countries for these compounds. Hites[173] examined river waters by computerized GLC–high-resolution

mass spectrometry and found several plasticizers, trichlorobenzene, butyl benzoate, and biphenyl at levels of 0.1–30 ppb. GLC conditions included a 150-cm × 0.32-cm column of 0.05% OV-17 on glass beads with a temperature program from 70 to 250°C at 12°C/min. Hahn and Thier[174] determined biphenyl and *o*-phenylphenol in citrus fruits as their bromo-derivatives. The residues were extracted and subsequently reacted with bromine–iodine solution prior to GLC-ECD on a 1.5-m glass column packed with 1.5% QF-1/1% DC-200 (or 2.5% XE-60) on Chromosorb G AW DMCS operated at 200°C. The method was suitable for routine analysis at the 2- and 4-ppm levels, respectively (*ca.* 2 ng absolute sensitivity for each fungicide).

The TLC and HSLC of the fungicides biphenyl and *o*-hydroxybiphenyl and other hydroxybiphenyl metabolites was reported by Cassidy *et al.*[175]. Dansylated hydroxybiphenyls were separated on silica gel thin layers with chloroform–benzene (1:1) mobile phase in the order *o,o'*-(most sorbed), *p,p'*-, *o*-, and *p*-hydroxybiphenyl. HSLC on 15- or 40-cm columns of silica gel (7–18 μ) packed by a balanced-density slurry technique was used to separate the *o*- and *p*-dansyl derivatives (hexane–chloroform, 9:1, solvent) and the *o,o'*- and *p,p'*-derivatives (hexane–chloroform, 7:3). Both UV and fluorescence detectors provided linear calibration curves from 1 to 300 ng. The fluorescence detector was more sensitive (*ca.* 0.1 ng at 3:1 signal-to-noise ratio) but resulted in a loss of column efficiency (band broadening). An earlier report[176] demonstrated reaction conditions for the dansylation reaction of hydroxybiphenyls and *in situ* thin-layer quantitation between 5 and 500 ng in rat urine. TLC was carried out on silica gel layers with two solvent systems (Table 9). After development, the dried plates were sprayed with triethanolamine and evaluated with a Zeiss PMQ II Spectrometer, and identities of residues confirmed by mass spectrometry.

A thin-layer fluorescence method for detection of biphenyl and *o*-phenylphenol in foods was described[177]. After diethyl ether extraction, the compounds were separated on silica gel with hexane–ethanol–chloroform (97:2:1) and detected in 254-nm UV light. Respective R_F values were 0.70 and 0.25. Corneliussen[178] determined biphenyl in citrus fruits (10–100 ppm) by separation of residues with a steam liquid–liquid extraction and cleanup of the *n*-heptane extract by silica gel preparative TLC. The plate was developed with heptane and biphenyl detected under UV light as a bright blue spot on the yellow background of the fluorescent layer. Spots were removed from the layer and extracted with methanol for quantitative spectrophotometry at 248 nm.

A Varian HSLC detector was modified to improve stability and sensitivity by

TABLE 9

R_F VALUES FOR HYDROXYBIPHENYL-DANSYL DERIVATIVES

Solvents: I = benzene–chloroform (1:1); II = acetone–hexane (3:7).

Dansyl derivative	R_F	
	I	*II*
4-Hydroxybiphenyl*	0.58	0.62
2-Hydroxybiphenyl	0.47	0.62
4,4'-Dihydroxybiphenyl	0.20	0.48
2,2'-Dihydroxybiphenyl	0.10	0.45

* Equivalent to *p*-hydroxybiphenyl.

Callmer and Nilsson[179], who demonstrated linear calibration curves from 0.4 to 1000 ng biphenyl when chromatography on a 2.4-mm × 400-mm column packed with 0.88% 1,2,3-tricyanoethoxypropane on Corasil I support with isooctane mobile phase was used. Biphenyl eluted in 2 min at a flow-rate of 80 ml/h. Reeder[180] developed HSLC methods for the quantitation of biphenyl, thiabendazole, and *o*-phenylphenol in citrus products using 20-μ silica gel and three different solvents. These fungicides were detected by UV absorption with limits of less than 1 ppm.

E. Carboxin

Sisken and Newell[181] determined residues of carboxin (Vitavax) and its sulfoxide in seeds and seed oils by methanol extraction, partition cleanup of the extract, reductive hydrolysis to liberate aniline, distillation of aniline, and N-selective microcoulometric GLC. An 18-ft. 4% Carbowax 20M column, temperature programmed from 100 to 200 °C, eluted with hydrogen carrier gas (40–55 ml/min depending on column temperature), gave a retention time of about 15 min for the aniline peak from carboxin and its sulfoxide. The method was sensitive to < 0.2 ppm carboxin (60 ng aniline).

Chin *et al.*[182] used TLC on silica gel Chromagram sheets to study the degradation of carboxin in water and soil and its metabolism by barley and wheat plants. Five different TLC solvents were studied, and R_F values evaluated by spraying a 0.05% fluorescein methanolic solution and observation under 254-nm UV light or by performing radioautography. The solvents with R_F values of carboxin were methanol (0.6), 20% methanol–acetone (0.9), chloroform (0.8), benzene (0.1), and acetone (0.9). Tripathi and Bhaktavatsalam[183] detected carboxin and oxycarboxin on silica gel G layers with silver nitrate–bromophenol blue and potassium permanganate–sulfuric acid chromogenic reagents. The former reagent detected only carboxin as a blue spot at 2-μg levels while the latter detected 3-μg amounts of both fungicides as white spots on a pink background. R_F values were 0.8 and 0.4 for carboxin and oxycarboxin, respectively, when development was with chloroform. This same solvent was reported earlier by Allam and Sinclair[184], who detected the fungicide spots under UV light on a layer having a fluorescent indicator.

F. Dinocap

Karathane pesticide is a mixture of dinitrooctylphenyl crotonates, dinitrooctyl phenols, and mononitrooctyl phenols, of which dinocap is one component. Karathane formulations have been examined by various workers using GLC. Clifford *et al.*[185] and Clifford and Watkins[186] used columns containing mixed phases of diethylene glycol adipate polyester plus H_3PO_4. Boggs[187] reported a double peak for dinocap on a 10% DC-200 column used for the GLC-ECD of a series of dinitro herbicide methyl ethers. Kurtz and Baum[188] and Kurtz *et al.*[189] used columns packed with 3% QF-1 silicone oil on 60–80 mesh Gas-Chrom Q programmed from 100 to 230°C and an FID.

Dinocap was determined in formulations with TLC by Chiba and Yatabe[190]. Silica gel layers were developed with hexane–acetone (8:2), the dinocap spot was eluted with benzene and determined photometrically at 430 nm.

Chromatographic determination of dinocap residues has not been reported.

G. Dithianon

Eisenbeiss and Sieper[191] demonstrated the HSLC of this fungicide on Perisorb A porous-layer bead adsorbent with a Zeiss PMQ II 254-nm detector modified for column liquid chromatography and heptane–ethyl acetate (96.5:3.5) as carrier liquid. Five nanograms were the minimum detectable amount and linear calibration curves between 50 and 2000 ng were illustrated. Apples were analyzed after extraction and cleanup at 0.02- and 0.1-ppm levels with 80% recoveries.

H. DNOC

This phenolic compound, which acts as a fungicide, insecticide, and herbicide (ammonium salt), has been determined in urine along with PCP, several phenoxy acid herbicides, and a series of halo- and nitrophenol metabolites of organophosphate pesticides by a GLC-ECD method[192]. After extraction with diethyl ether, the phenols were ethylated with diazoethane, and the ethers chromatographed on a 2-g silica gel column (2% water). PCP was eluted in the first fraction with 8 ml of 20% benzene–hexane, and DNOC in the third through fifth fractions (10 ml each of 60% benzene–hexane, 80% benzene–hexane, and straight benzene). Chromatographic analysis at the 0.01- to 0.1-ppm level was made on a 4% SE-30/6% OV-210 column at 175°C, with a sensitivity of 0.01–0.3 ng for the individual phenols.

Phenolic pesticides are either converted to corresponding ethers or tailing is avoided by gas chromatographing the free phenols on low-loaded polar phases in the presence of H_3PO_4 at relatively low column temperatures. DNOC had the following retention times (min) on 80-cm × 3-mm columns packed with 3% of each following phase plus 1% H_3PO_4 at 200°C and a carrier flow-rate of 1.0 kp/cm^2: neopentyl glycol succinate (0.82), cyclohexanedimethanol succinate (1.04), butanediol succinate (0.93)[193].

Free DNOC had an R_F value of 0.83 on a thin layer of cellulose impregnated with mineral oil–acetic acid–diethyl ether (5:2:93) and developed with methanol–acetic acid–water (73:2:25). The methyl ether had an R_F value of 0.87 on mineral oil-impregnated cellulose developed with methanol–acetonitrile–water (30:25:45). Detection at 0.05 μg (free) and 0.1 μg (ether) was made by spraying with stannous chloride–dimethylaminobenzaldehyde reagent to produce yellow-orange fluorescent spots[194]. Guardigli *et al.*[195] identified and quantitated DNOC by TLC in various crops at a sensitivity below 0.05 ppm (0.5-μg detection). After extraction and cleanup by alkaline hydrolysis and liquid–liquid partition, residues were converted to nitro derivatives by reaction with 2% $NaNO_2$ in concentrated H_3PO_4. The derivative was developed on silica gel with benzene–acetic acid (85:15) and detected by reduction of the nitro group to the corresponding amine followed by diazotization and coupling with Bratton–Marshall reagent.

I. Ethirimol and dimethirimol

Bratt *et al.*[196] elucidated the metabolism of the systemic pyrimidine fungicide dimethirimol by rats and dogs with the aid of radio-TLC. Silica gel GF layers were developed with the listed solvents and compounds detected by viewing under 254-nm

UV light: (a) water-saturated butanol (R_F dimethirimol = 0.54), (b) *n*-butanol–acetic acid–water (4:1:5) (0.48), (c) *n*-butanol saturated with 3 *N* ammonia (0.72), (d) chloroform–methanol (9:1) (0.71). Two-dimensional chromatography with solvents (d) and (c) in turn separated nineteen urinary metabolites and solvents (a) and (b) resolved thirteen metabolites.

Bagness and Sharples[197] determined ethirimol and dimethirimol in technical and formulated materials by GLC-FID and quantitative TLC. For GLC, the fungicides were converted into volatile trimethylsilyl ethers and chromatographed on a 5-ft. × 4-mm column of 10% E-301 on silanized 100–200 mesh Celite. The retention of ethirimol relative to *n*-nonadecane internal standard was 0.53 at 220°C while that of dimethirimol relative to *n*-octadecane was 0.47 at 200°C. TLC was carried out on silica gel GF_{254} layers developed with chloroform–acetone–acetic acid (75:10:15) for ethirimol and methanol–dichloromethane (1:9) for dimethirimol. The compounds were located as quenched zones under UV light, adsorbent bands were removed by the "vacuum-cleaner" technique, and methanol extracts were analyzed by UV absorption spectroscopy at 297 and 303 nm, respectively.

J. Fentin acetate and fentin hydroxide

These compounds, which are active ingredients in the fungicides Brestan and Du-Ter, and their degradation products have been analyzed after photolysis *in vitro* and in soils by Cenci and Cremonini[198]. Samples were cold extracted with 95% ethanol–ethyl acetate (1:1) and extracts chromatographed on silica gel H layers with *n*-butanol–ethanol–water (4:2:1). Both compounds yielded three spots with R_F values of approximately 0.0, 0.45, and 0.88. The former two were detected with 0.5% aqueous catechol violet and the third with aqueous vanadophosphoric acid at 0.05-μg levels.

Tin was determined in organic material, after application of tin-containing fungicides to plants, by wet ashing, extraction with cupferron into chloroform, oxidation of the cupferron complex with HNO_3–$HClO_4$, and radial PC with 3 *N* HCl–saturated *n*-butanol as solvent to separate tin from other metals. The tin-containing chromatographic zone was wet ashed and the phenylfluorone complex photometrically estimated at 546 nm in acid solution[199].

K. o-Phenylphenol (see also Sections 7D and 7L)

Davenport[200] reported a TLC method for determination of *o*-phenylphenol residues in acetonitrile extracts of fruits and vegetables at 0.1 to 200 ppm which was satisfactorily validated at 0.5- to 10-ppm levels by interlaboratory studies. Extraction and Florisil column cleanup were carried out as described by Porter *et al.*[201] for carbaryl residues in fruits and vegetables. The cleaned-up extract was evaporated, applied to an aluminum-backed EM silica gel layer, and developed along with 0.05- to 0.5-μg standard spots using benzene as solvent. The layer was sprayed with 1 *N* alcoholic KOH followed by *p*-nitrobenzenediazonium fluoborate chromogenic reagent to produce a pink spot with R_F 0.5–0.8 for the fungicide. Semiquantitation was based on visual comparison of sample and standard spot sizes and intensities. Folpet residues, if also present, would interfere with the *o*-phenylphenol spot, in which case

development was carried out with hexane–ethyl acetate (2:1) to separate the two fungicides. Other fungicides detected by the chromogenic reagent include captan (salmon-orange, 25 μg detected), folpet (yellow-orange, 25 μg), and DNOC (yellow, > 100 μg).

L. Thiabendazole (see also Section 7B)

In a method by Hey[202] for analysis of citrus fruits and bananas, samples were reflux-extracted for 3 h with dichloromethane and concentrates cleaned-up on silica gel GF_{254} plates with chloroform and benzene–acetic acid–acetone–water as solvents. Spots located under UV light were removed, extracted with methanol, and determined by UV spectrophotometry or GLC of silyl or methyl derivatives on a 5% SE-30 column at 200 °C with flame ionization or nitrogen-specific detectors. A rapid TLC screening method reported by Reinhard[203] detected the fungicides thiabendazole, biphenyl, *o*-phenylphenol, and diphenylamine in peels of citrus fruits at 1- to 2-μg levels. Extraction was made in turn with alkaline and then acidic dichloromethane, followed by silica gel TLC and detection under UV light or by spraying with Dragendorff's reagent for thiabendazole, or with LeRosen's reagent and spraying with HNO_3 for the others.

A TLC-spectrophotofluorimetric method was reported by Norman *et al.*[204] for quantitation of thiabendazole on and in citrus fruits from 0.2 to 6 ppm. TLC ways carried out on fluorescent, aluminum-backed alumina layers developed with chloroform–methanol (98:2) after extraction of residues with ethyl acetate and partition cleanup, if required. Detection down to 0.2 μg was made under short-wave UV light, and the purple, absorbing spot was eluted by cutting out the spot area and immersing in methanol–0.1 *N* HCl (99:1) for 30 min.

Kroeller used TLC to assay tobacco smoke condensate[205] and orange peels[206] for thiabendazole. In the former method, cleanup by chloroform extraction and steam distillation preceded silica gel TLC with benzene–acetic acid–acetone–water (10:4:1:0.4) solvent. Residues were extracted with dichloromethane from orange peel, cleanup was by washing with 0.1 *N* HCl and then 0.1 *N* NaOH, and chromatography was on alumina layers with benzene–acetone–water solvent. In each case the fungicide was detected under UV light, eluted, reacted with *p*-phenylenediamine, and the resulting blue color evaluated by spectroscopy. The limit of detection was 0.1 ppm in orange peels.

M. Thiophanate (see also Section 8)

The translocation of the fungicides MBC (degradation product of benomyl) and thiophanate-methyl as affected by plant nutrition was studied by Al-Adil *et al.*[207]. TLC was carried out on polyamide 11-F sheets with chloroform–ethyl acetate–acetic acid (190:10:4) solvent for MBC and silica gel F sheets with chloroform–methanol (9:1) for thiophanate-methyl. Spots were located under UV light and mapped by autoradiography. Soeda *et al.*[208] performed TLC of thiophanate-methyl and its metabolites on Eastman silica gel Chromagram sheets developed in unsaturated tanks with ethyl acetate–hexane–acetic acid (20:80:2) as solvent. The compounds were detected under 254-nm UV light, thiophanate-methyl having an R_F value of 0.3.

Other solvents employed for metabolite separations were ethyl acetate–hexane (saturated tank) and ethyl acetate–chloroform–acetic acid (10:90:2, unsaturated tank).

N. Tutane

Day *et al.*[209] analyzed *sec.*-butylamine residues in certain fruit samples at 2.0 ppm by steam distillation of the amine from the tissue, removal of interferences by a carbon tetrachloride wash, reaction with 1-fluoro-2,4-dinitrobenzene, and GLC-ECD analysis on a 6-ft. × ¼-in. 2% DEGS column at 188°C. With a 80 ml/min carrier gas flow-rate, the derivative (N-*sec.*-butyl-2,4-dinitroaniline) had a retention time of 8 min. In some cases, cleanup by TLC prior to GLC was required. For this, silica gel G layers and hexane–diethyl ether (7:3) solvent were employed. The area of adsorbent containing the DNP derivative, as indicated by a guide zone of pure standard developed on the same plate, was removed, the derivative was eluted with chloroform, and the solvent was exchanged for benzene prior to GLC analysis.

8. MIXED FUNGICIDES

The following systemic fungicides and metabolites were separated and detected by Von Stryk[210] on Eastman Chromagram silica gel sheets containing fluorescent indicator: benomyl, thiophanate, thiophanate-methyl, MBC, benzimidazole, and 2-aminobenzimidazole. Solvent systems for the two-dimensional separation were benzene–methanol (9:1) followed by ethyl acetate–chloroform (6:4). One-dimensional development with the first of these solvents separated all compounds except MBC and benzimidazole, while the second resolved all but the thiophanate isomers. All compounds were visible under 254-nm UV light as dark spots on a pink background,

TABLE 10

TLC OF SYSTEMIC FUNGICIDES[211]

Fungicide	*Detection method*	*Detection limit (μg)*	*Spot position***
Dimethirimol	color*	0.6	CBBA
Ethirimol	color*	0.6	BBAA
Thiabendazole	UV	1.0	BBAB
Benomyl	UV	0.8	DCCC
MBC	UV	NR***	CBAA
Carboxin	UV	0.5	DCBC
Oxycarboxin	UV	0.5	BCAA

* Potassium iodobismuthate spray followed by exposure to bromine vapor.

** Spot locations for chromatographic systems 1–4, in order, where A = R_F 0.0, B = 0.25–0.50, C = 0.50–0.75, D = 0.75–1.0. Systems 1–3: silica gel 60 F_{254} adsorbent developed with diethyl ether–glacial acetic acid–methanol (100:5:2), acetone, and light petroleum (60–80°C)–acetone (3:1, double development), respectively. System 4: aluminum oxide F_{254} neutral (Type E) adsorbent developed with diethyl ether–methanol (40:1).

*** None reported.

and MBC was selectively detected as a blue spot at a level as low as 25 ng by N-2,6-trichloro-*p*-benzoquinoneimine reagent. However, this latter result was not reproduced by Baker *et al.*[211], who studied the TLC of a group of systemic fungicides and MBC. The results of these latter workers are shown in Table 10. All compounds could be separated by use of these four systems. These TLC separations were combined with a bioassay technique to detect and identify thiabendazole, benomyl, MBC, and thiophanate-methyl on citrus fruit skin at 0.5- to 2-ppm levels[212]. Minimum amounts of the fungicides listed in Table 10 and thiophanate-methyl detectable as standards were 0.05–10 μg, using a fungal growth inhibition procedure.

Fishbein[213] studied the TLC of some sixty isomeric halo- and nitro-derivatives of aniline and benzene including several fungicides. Silica gel DF-5 chromatoplates activated at 75°C were developed by the ascending method with (A) 2.5% ethyl alcohol in benzene, (B) 20% ethyl acetate in benzene, and (C) 2.5% acetone in benzene. After evaporating the solvent, spots were located under 254-nm UV light and by DDQ, TCNE, and Gibb's reagents. $R_F \times 100$ values of the fungicides studied were as follows:

	A	*B*	*C*
DCNA	41	67	66
PCNB	70	66	71
TCNB	63	56	65
o-Dichlorobenzene	64	65	63
m-Dichlorobenzene	63	60	63
p-Dichlorobenzene	63	62	65

9. APPENDIX

NAMES, CHEMICAL NAMES, AND ALTERNATE NAMES OF FUNGICIDES[214]

*Name**	*Chemical name*	*Alternate name**
BAS-3191	2,5-dimethyl-3-furancarboxylic acid anilide	
benomyl	methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate	Benlate
binapacryl	2-*sec*.-butyl-4,6-dinitrophenyl-3-methyl-2-butenoate or 2-(1-methyl-*n*-propyl)-4,6-dinitrophenyl-2-methylcrotonate	Morocide
biphenyl	diphenyl, phenylbenzene	
Brestan	triphenyltin acetate	fentin acetate, Cercostan
Bulbosan	1,3,5-trichloro-2,4,6-trinitrobenzene	
captafol	N-(1,1,2,2-tetrachloroethylthio)-3α,4,7,7α-tetrahydrophthalimide	Difolatan

* Trade names are capitalized while common names are not, except for abbreviations such as PCNB.

Name	*Chemical name*	*Alternate name*
captan	N-(trichloromethylthio)-3α,4,7,7α-tetrahydrophthalimide	Merpan, Orthocide 406
captax	2-mercaptobenzothiazole	
carboxin	5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide	Vitavax
Cela W 524	N,N′-bis(1-formamido-2,2,2-trichloroethyl)piperazine	triforine
Cerenox	quinoneoximebenzoyl hydrazone	
Ceresan	methoxyethylmercuric chloride	
chloranil	2,3,5,6-tetrachloro-1,4-benzoquinone	Spergon
chloroneb	1,4-dichloro-2,5-dimethoxybenzene	Demosan
chloronitropropane	1-chloro-2-nitropropane	Korax, Lanstan
chlorothalonil	2,4,5,6-tetrachloroisophthalonitrile	Bravo, Daconil 2787
dazomet	tetrahydro-3,5-dimethyl-2H-thiadiazine-2-thione	Mylone
DCNA	2,6-dichloro-4-nitroaniline	Botran, dichloran
dichlofluanid	N,N-dimethyl-N′-phenyl-(N′-fluorodichloromethylthio)sulfamide	Euparen, Elvaron
dichlone	2,3-dichloro-1,4-naphthoquinone	Phygon
dichlozoline	3-(3,5-dichlorophenyl)-5,5-dimethyloxazolidinedione-2,4	Sclex
dimethirimol	2-dimethylamino-4-hydroxy-5-*n*-butyl-6-methylpyrimidine	Milcurb
dinobuton	2-(1-methyl-2-propyl)-4,6-dinitrophenyl isopropylcarbonate	
dinocap	2,4-dinitro-6-(1-methylheptyl)phenyl crotonate	Karathane, Arathane
Dithane M-45	a coordination product of Zn^{2+} and manganese ethylenebisdithiocarbamate, related to maneb and zineb	
dithianon	1,4-dithiaanthroquinone-dicarbonitrile-2,3	Thynon
DNOC	4,6-dinitro-*o*-cresol (2-methyl-4,6-dinitrophenol)	
dodine	*n*-dodecylguanidine acetate	Cyprex
drazoxolone	4-(2-chlorophenylhydrazono)-3-methyl-5-isoxazolone	
Du-Ter	triphenyltin hydroxide	fentin hydroxide, TPTH
Dyrene	2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine	anilazine
ethirimol	2-ethylamino-4-hydroxy-5-*n*-butyl-6-methylpyrimidine	
ferbam	ferric dimethyldithiocarbamate	

Name	*Chemical name*	*Alternate name*
folpet	N-(trichloromethylthio)phthalimide	Phaltan
fuberidazole	2-(2′-furyl)-benzimidazole	Voronit
Germisan	phenylmercury pyrocatechol	
glyodin	2-heptadecyl-2-imidazoline acetate	
HCB	hexachlorobenzene	
hexachlorophene	2,2′-methylene bis(3,4,6-trichlorophenol)	Nabac
maneb	manganese ethylenebisdithiocarbamate	
Memmi	N-methylmercuri-1,2,3,6-tetrahydro-3,6-methano-3,4,5,6,7,7-hexachloro-phthalamide	
Merthiolate	sodium ethylmercury thiosalicylate	Thimerosal, Elcide
nabam	sodium ethylenebisdithiocarbamate	
Panogen	cyano(methyl mercuri)guanidine	Morsodren, Panodrin A-13
parinol	α,α-bis(*p*-chlorophenyl)-3-pyridinemethanol	Parnon
PCNB	pentachloronitrobenzene	quintozene, terrachlor
PCP	pentachlorophenol	
o-Phenylphenol	2-phenylphenol	
Polyram	a mixture of ethylenebis(dithiocarbamate)-zinc and [dithiobis(thiocarbonyl)imino-ethylene]bis(dithiocarbamato)zinc	
Quinex	phenylmercury oxyquinolate	Ortho LM
Quinomethionate	6-methyl-2,3-quinoxalinedithiol cyclic *S,S*-dithiocarbonate	Morestan, oxythioquinox
TCNB	1,2,4,5-tetrachloro-3-nitrobenzene	Fusarex, Folosan, tecnazene
tetraiodoethylene	1,1,2,2-tetraiodoethylene	
thiabendazole	2-(4′-thiazolyl)-benzimidazole	
thiophanate	1,2-bis(3-ethoxycarbonyl-2-thioureido)-benzene	Topsin
thiophanate-methyl	1,2-bis(3-methoxycarbonyl-2-thioureido)-benzene	
thiram	bis(dimethylthiocarbamoyl) disulfide	
triarimol	α-(2,4-dichlorophenyl)-α-phenyl-5-pyrimidinemethanol	
trichlorophenol	2,4,5-trichlorophenol and 2,4,6-trichloro-phenol	Dowcides 2 and 2S
Tutane	*sec.*-butylamine or 2-aminobutane	
Urbazit	methylarsinebisdimethyldithiocarbamate	
Vapam	sodium N-methyldithiocarbamate	metham
Zineb	zinc ethylenebisdithiocarbamate	
Ziram	zinc dimethyldithiocarbamate	

10. SUMMARY

The separation and analysis of a wide range of fungicides by gas, liquid, column, paper, and thin-layer chromatography have been reviewed. Major attention has been given to methods for the identification and quantitation of individual and multiresidues of fungicides in environmental and agricultural samples.

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PUBLICATION SCHEDULE FOR 1975

Journal of Chromatography (incorporating *Chromatographic Reviews*)

MONTH	D 1974	J	F	M	A	M	J	J	A	S	O	N	D
JOURNAL	101/1 101/2 102	103/1 103/2 104/1	104/2 105/1 105/2	106/1 106/2	107/1 107/2	108/1 108/2	109/1 109/2	110/1 110/2	The publication schedule for the volumes 111, 112 and 114 will be published later.				
REVIEWS*				113/1					113/2			113/3	

* Volume 113 will consist of *Chromatographic Reviews*. The issues comprising this volume will not be published consecutively, but will appear at various times in the course of the year.

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2 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968, p. 201.
3 R. D. Marshall and A. Neuberger, in A. Gottschalk (Editor), *Glycoproteins*, Vol. 5, Part A, Elsevier, Amsterdam, 2nd ed., 1972, Ch. 3, p. 251.

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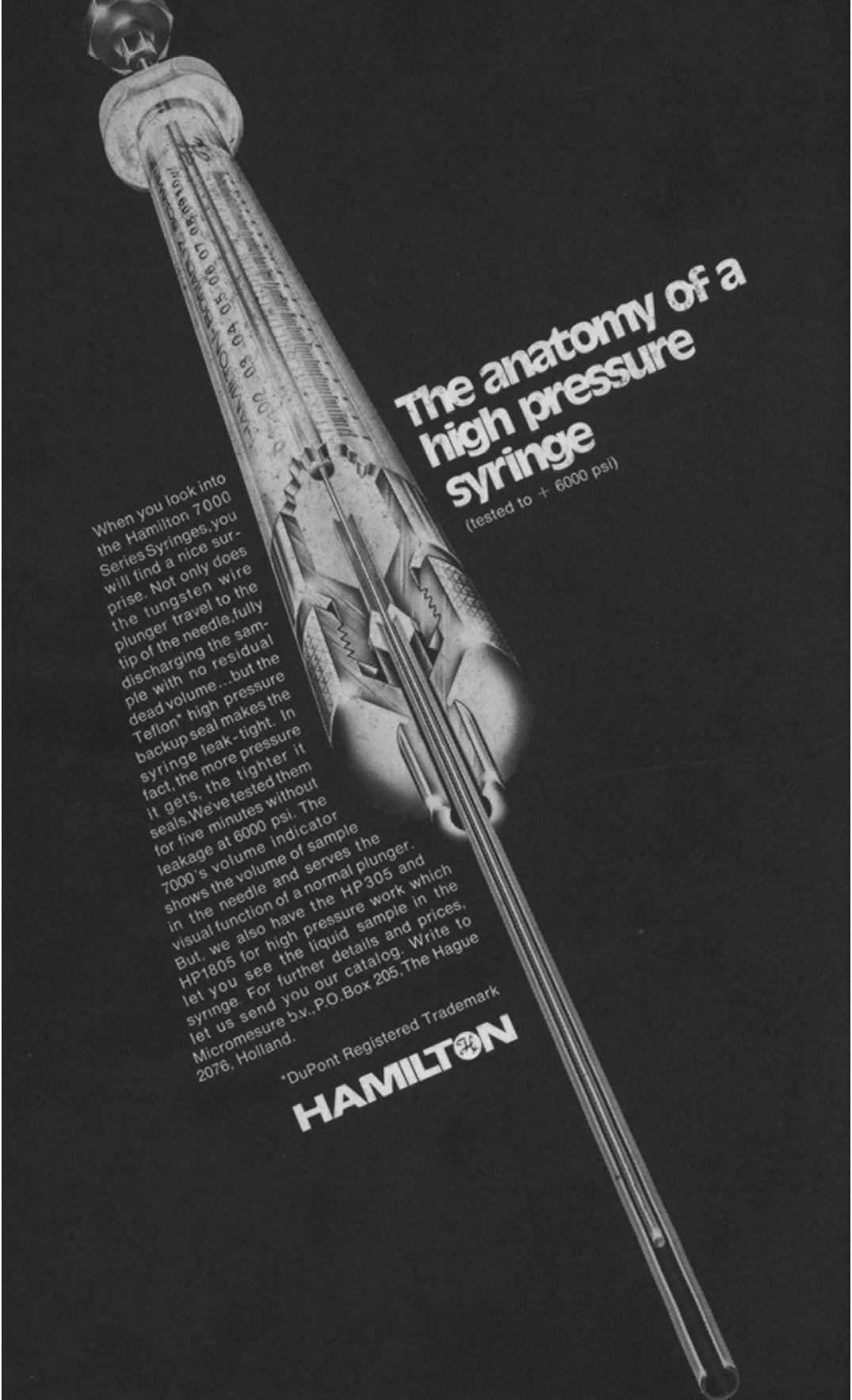

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