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CHROMATOGRAPHY IN STUDIES OF QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIPS

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CONTENTS

1. INTRODUCTION

In 1975 Tomlinson[1] published an extensive review on the application of thin-layer chromatography (TLC) to the correlation analysis of quantitative structure–activity relationships (QSAR). Another paper was published by Bhutani[2]. Although fundamentally Tomlinson's remarks still remain valid (and are not repeated here), numerous papers have subsequently been published in which new approaches and modern techniques are described. At present, chromatography is routinely, and perhaps mechanically, used by medicinal chemists. In such a situation it seems worthwhile to bring together more important recent achievements in the field.

Chromatography is most widely applied in the Hansch approach to QSAR based on linear free energy relationships[3]. It should be noted that an analogous approach to the evaluation of substituent contributions to chromatographic retention, quantitative structure–retention relationships, has recently been proposed by Chen and Horváth[4]. The Hansch approach consists of the assumption that, to a first approximation, the free energy change in a standard biological response is a linear sum of individual energetic contributions, namely hydrophobic, electronic and steric.

The change in the free energy of a biological response due to the hydrophobic nature of the drug can be represented by the logarithm of the oil–water partition coefficient or quantities related to it. The electronic term is usually expressed by means of Hammett's constants or dipole moments. Expressing the steric effect in a

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linear free energy relationship has long been a problem. Usually Taft's steric constant, E_s, and molar volume are used.

2. HYDROPHOBICITY AND CHROMATOGRAPHIC PARAMETERS

Most of the papers published on chromatography in QSAR concern hydrophobicity changes. That part of the free energy change which can be attributed to hydrophobic bonding usually gives the major contribution to the biological response compared with the electronic and steric terms. Hydrophobic interactions in a chromatographic process can be separated from other types of intermolecular bonding.

The hydrophobic nature of a drug might be represented by the logarithm of the partition coefficient (log P) obtained from studies of the distribution of the drug between an immiscible polar and a non-polar solvent pair. The recommended solvent system for measurements of partition coefficients is 1-octanol–water. This reference system was chosen[5,6] as reflecting the partition between lipid membranes and extracellular fluids.

Following Green and Marcinkiewicz[7,8], who found that ΔR_M is additive for a number of benzenoid compounds, Iwasa *et al.*[9] suggested usefulness of chromatographic data for QSAR. At the same time, Boyce and Milborrow[10] published the paper in which they correlated molluscicidal activity of a series of N-*n*-alkyltritylamines with R_M data. In 1974 Haggerty and Murrill[11] suggested the utility of reversed-phase high-performance liquid chromatography (HPLC) for the determination of partition coefficients. The logarithm of the capacity factor, log k', the quantity measured by HPLC, is analogous to R_M in flat-bed chromatography.

At first, the partition coefficient was considered as a measure of the ability of a given compound to penetrate through various biological membranes. In other words, it was considered as a measure of the probability that a given molecule would reach its site of action in a given time interval. Another mechanistic interpretation of the meaning of partitioning properties is based on the concept of the hydrophobic bond formed between the drug and a hydrophobic protein receptor.

Although the choice of 1-octanol as a compound reflecting the properties of the lipid components of the cell membrane has been questioned, the large number of 1-octanol–water partition data collected by Hansch's group has made 1-octanol a common reference standard. Fujii *et al.*[12], in studies on 15 compounds of a series of C_{16}, C_{18} and C_{20} fatty acids and their esters and alcohols, found linoleic and palmitic acids to be the best for reproducing the fatty acid components of the cell membrane. However, the differences among the compounds studied have been of little importance for the quality of QSAR concerning the antistaphylococcal activity of ω-amino acids and their L-histidine dipeptides. This is in agreement with Collander's[13] finding, further developed by Leo and Hansch[14] and Leo *et al.*[6], that rectilinear relationships exist between partition coefficients found in one system and those found in a second system, provided that the polar phase is water and the non-aqueous phases are similar.

Measurement of log P by the conventional "shake flask" method is tedious. It is difficult to determine log P for compounds that are poorly soluble in water or that cannot be detected by conventional methods. Instead of measuring log P values by equilibration methods, partition chromatographic data can be used. According

to Tomlinson[1], the main advantages of chromatographic methods over direct partition methods for obtaining an index of hydrophobicity are that they are simple to use, rapid and less tedious, little material needs to be used, they are able to accomodate drug molecules with very high or low log P value (such solutes require a long equilibration in normal "shake flask" methods), the material need not be ultrapure, there is no need for specific quantitative analysis of the solute and more reproducible results are usually obtained.

2.1. Conditions of chromatographic determination of hydrophobicity

2.1.1. Control of partitioning mechanism

It is important to make chromatographic determinations in systems where partition either is the sole process taking place or it predominates over others. Unfortunately, in many recent papers concerning chromatographic determinations of lipophilicity the partition mechanism has been assumed but not, however, always proved.

Partition chromatography can be carried out by means of TLC, paper chromatography and HPLC. Reversed-phase techniques are commonly applied, although a few successful biological correlations have been obtained using the direct technique in paper chromatography[15] and TLC[16]. As hydrophobic stationary phases, silicone oil, 1-octanol, oleyl alcohol, liquid paraffin and chemically bonded phases, *e.g.*, ODS, are usually used. ODS is commonly used for reversed-phase HPLC, but Siouffi *et al.*[17], based on extensive studies, proposed a practical approach to the application of bonded phases in TLC.

To check whether the R_F values of compounds in reversed-phase chromatography are determined exclusively by partitioning between the stationary phase and the mobile phase, Hulshoff and Perrin[18] employed the relationship

$$1/R_F = 1 + {}_sP^x k C_{ol} \tag{1}$$

where ${}_sP^x$ is the partition coefficient in the system oleyl alcohol/methanol–water, analogous to a chromatographic system, k is a constant and C_{ol} is the oleyl alcohol concentration in the mixture used for impregnation of the plates. When no adsorption of the compounds (benzodiazepines) on to the support phase during the migration took place, straight lines were obtained when $1/R_F$ was plotted against C_{ol}, with intercepts close to unity and slopes of ${}_sP^x k$. To avoid interaction of the compounds being chromatographed with free silanol sites on bonded ODS supports, silylation with hexamethyldisilazane (HMDS) or trimethylsilyl chloride (TMSCl) has been suggested[19]. However, a few of the silanol sites on chemically bonded phases will always be sterically protected from silylating reagents. As is evident from recent studies by Knox *et al.*[20], electrostatic interactions then occur, resulting in marked exclusion of anionic species due to Donnan equilibria. Donnan exclusion may be reduced by increasing the ionic strength of the mobile phase.

When one relates the partition chromatographic R_M value to the logarithm of the partition coefficient, log P_s, determined in a system identical with the chromatographic system, one should obtain Martin's relationship:

$$R_M = \log P_s + \log V_s/V_M \tag{2}$$

where V_s and V_M are volumes of the stationary and mobile phases, respectively. In practice, the slope of eqn. 2 deviates slightly from unity. This may be explained according to Bird and Marshall[21] as resulting from difficulties in adjusting ionic strengths in the "shake flask" and chromatographic processes to exactly the same value. Generally, a slope of exactly unity could be expected only if the two chromatographic phases were precisely identical with the two phases in the classical "shake-flask" experiment[22].

2.1.2. Choice of the partitioning system

Chromatography with pure water as the mobile phase is not always possible owing to too low or too high a solute polarity. Accuracy in TLC demands $0.2 < R_F < 0.8$ (ref. 23). This gives a range of R_M of less than 1.5 decades, whereas HPLC is capable of 3–4 decades[24]. In such a situation it is often necessary to change composition of the mobile phase in order to obtain reliable R_M values when one deals with a series of compounds of various polarity. Acetone and ethanol are the most popular organic modifiers of the aqueous mobile phase. R_M values of all compounds of a series for any fixed organic solvent concentration in the mobile phase can be used for QSAR purposes. However, the R_{M_w} values are commonly used, which are obtained by extrapolation to a 100% water concentration in the mobile phase. Such an extrapolation is generally valid as R_M varies linearly with the volume fraction of organic solvent in the aqueous mobile phase as represented by the Soczewiński–Wachtmeister equation[25]:

$$R_M = R_{M_w} + bC \tag{3}$$

where R_{M_w} is the value of R_M for pure water as the mobile phase, b is a constant and C is the concentration of organic modifier in the mobile phase.

2.1.3. Effect of ionization of solute

Special care must be taken if one deals with ionizable compounds. The effects of ionization are unfortunately ignored in many new methods for the determination of partition coefficients[26]. The reference partition coefficient, P, refers to the ratio of neutral, un-ionized compound in each phase. What one actually observes are the distribution coefficients. The distribution coefficient is defined[27] as the ratio of the concentration of a compound in the organic phase to the concentration of all species (neutral and ionized) in the aqueous phase at a given pH. The organic phase is assumed to contain only un-ionized species, which is never true as there may be some solution of an ionized compound in the hydrophobic phase as an ion pair with the buffer ions. If one knows the effects of ionization, one can simply use the distribution coefficient, D, in place of the partition coefficient, P, but one must correct for relative differences in hydrogen bonding effects when comparing different experimental procedures[28]. The reference partition coefficient, P, of neutral species is

$$P = D/(1 - \alpha) \tag{4}$$

where α is degree of ionization. Thus, for acids

$$P = D\left(\frac{K_a}{H} + 1\right) \tag{5}$$

where K_a is the dissociation constant and H is the hydrogen ion concentration. Unger *et al.*[26] rearranged eqn. 5 into the form

$$D = P + K_a(-D/H) \tag{6}$$

Correlating the distribution coefficient, D, or the capacity factor, k' from HPLC, with $-D/H$ for acids or $-DH$ for bases, they determined the intercept P and the slope K_a (or $1/K_a{}^b$ for bases). To determine it most accurately, measurements should be made near pH 2 and approximately the pK_a for acids and near pH 8 and the $pK_a{}^b$ for bases. Unger *et al.*'s method is capable of determining simultaneously lipoid–aqueous partition, distribution and ionization coefficients (P, D and K_a, respectively), all of which are of value for QSAR purposes. The method has further been developed by Unger and Feuerman[29], based on the work by Horváth *et al.*[30], to model bulk phase partitioning for lipophilic acids, including ion-pair partitioning. The equation used has the form

$$\log D = \log (P + P'K_a/H) - \log (1 + K_a/H) \tag{7}$$

where P' is partition coefficient for the anion. The constants P, P' and K_a were derived from the non-linear least square fit of eqn. 7. Unger and Feuerman[29] supported Wahlund and Beijersten[31] in concluding that anions of lipophilic acids can be considerably more lipophilic than is commonly assumed and their partitioning should be taken into consideration for QSAR purposes.

2.2. Preparation of the partitioning chromatographic system

Much effort has been directed to preparing proper lipoid phases for partition chromatography. In TLC (or HPLC) silica gel, Kieselguhr G or microcrystalline cellulose have been impregnated by allowing a solution of a reversed-phase compound in a volatile solvent to cover the support. The solvent was then evaporated, usually with heating at 40–60°C. *n*-Hexane has commonly been used as a solvent for paraffin oil, after Boyce and Milborrow[10]. Fatty acids and their derivatives have usually been dissolved in diethyl ether[12] or toluene[32], 1-octanol in acetone[21,33] and silicone oil in ethanol[34]. The polar mobile phase has been saturated with the stationary phase substance. Hulshoff and Perrin[18,35] obtained Kieselguhr G impregnated with oleyl alcohol by coating glass plates with a slurry of Kieselguhr G in a mixture of oleyl alcohol, acetone and dioxan. A similar procedure was adopted by Kuchař *et al.*[36] for coating silica gel G with silicone oil, the volatile components of the impregnating solution being evaporated off within 16 h at 20°C.

When performing direct partition chromatography, usually paper chromatography or TLC on cellulose layers, a support is impregnated with a polar solvent, *e.g.*, formamide in ethanol[16].

Octadecyl chains chemically bonded to pellicular silica gel formed a hydrophobic phase used in the first HPLC determinations of partition coefficients[11,19,37]. McCall[19] blocked unbonded silanol sites present in commercial stationary phases by treating them with HMDS and TMSCl in hot pyridine. Mirrlees *et al.*[24] argued that the only true model for 1-octanol (the reference partitioning solvent) is 1-octanol

itself. According to them[24], a bonded ODS support must have essentially the partitioning characteristics of a hydrocarbon (there is no ideal correlation between 1-octanol and hydrocarbon partitioning). In such a situation, Mirrlees *et al.*[24] coated the column packing (Kieselguhr) with water-saturated 1-octanol. 1-Octanol-saturated water (buffered) was used as the eluent.

Miyake and Terada[38] described a method for preparing a 1-octanol-coated column that had high stability. Hot Corasil I was mixed with 1-octanol, the mixture was slurry packed and excess of 1-octanol removed by elution with buffer saturated with 1-octanol.

Unger *et al.*[25] prepared a 1-octanol-coated ODS column by injecting 1-octanol directly into the column under pressure until droplets appeared. The column was then flushed with 1-octanol-saturated buffer until the eluate appeared clear. As 1-octanol itself is very lipophilic it bonds strongly to ODS and gives a stable column. Moreover, 1-octanol is bonded to octadecyl chains with its hydroxyl end free to interact with the solute, as is expected in "shake flask" octanol–water partitioning.

Systems with adsorbed 1-octanol ideally reflect partitioning observed in equilibration methods. However, reversed-phase chromatography of very lipophilic compounds cannot be performed with 1-octanol as the stationary phase because the polar solvent concentration in the mobile phase, ncessary for obtaining measurable retention times, would solubilize 1-octanol[39]. For this reason, other stationary phases have been proposed. Hulshoff and Perrin[18] impregnated Porasil C and Chromosorb P NAW with oleyl alcohol by the solvent evaporation technique[40]. A similar technique was applied for coating pellicular Corasil II silica with squalene[41].

In the author's opinion modern commercial ODS columns, without any additional coating, give results that are precise enough for QSAR purposes (especially if one considers the low precision of the biological data involved) and are convenient over a wide range of operational conditions.

Recently Riley *et al.*[42], based on extensive studies on ion-pair reversed-phase HPLC, proposed a system using sodium dodecylsulphate as the pairing ion and methanol as the organic modifier for the determination of indices of hydrophobicity. They successfully applied their hydrophobicity indices to describe the antibacterial activity of a series of 1,3,5-*s*-triazines.

So far, little attention has been paid to the type of buffer used in partition experiments. However, Wang and Lien[43] reported recently that the true partition coefficients (corrected for ionization) of acidic and basic drugs obtained from different buffer systems could not be suitable for regression analysis without additional correction. According to the authors[43], for acidic and neutral drugs phosphate buffer (pH 7.4) appears to give corrected partition coefficients closer to the values obtained from the 1-octanol–water system than acetate and bicarbonate buffers. Therefore, the phosphate buffer system is probably the most suitable system for measurements of corrected partition coefficients for QSAR work, provided that the drug is soluble enough in this system. It should be emphasized that the possibility of precise pH control during the partitioning is a great advantage of HPLC over TLC. HPLC is also a rapid and reproducible technique.

The determination of partition coefficients of volatile substances is often very difficult and time consuming. For this reason, Boček[44] developed a gas–liquid chromatographic (GLC) method using water and oleyl alcohol as liquid stationary phases.

Boček assumed the partition coefficient of a compound in the system oleyl alcohol–water to be equal to the ratio of partition coefficients in the system oleyl alcohol (saturated with water)–nitrogen and water–nitrogen which could be determined by GLC. The method is complicated as care must be taken to eliminate the influence of adsorption and thus to determine true partition coefficients.

3. STERIC DATA CHROMATOGRAPHICALLY DETERMINED

Normally, GLC retention data are not related to the hydrophobic properties of the solute. During GLC separation Van der Waals and polar rather than hydrophobic interactions take place. The lack of correlation between GLC retention data and the hydrophobicity parameters has been shown by Steurbaut *et al.*[45].

Gas chromatography on nematic phases, however, can be utilized to obtain some information concerning the steric characteristics of the compounds being studied.

It has been reported by Kaliszan *et al.*[46] that the biochemical activity of polycyclic aromatic hydrocarbons (PAHs) depends quantitatively on the shape of the hydrocarbon molecules. The shape parameter has been numerically expressed by the ratio of the longer to the shorter sides of the minimum rectangular envelope around the structure, drawn proportionally to the atomic dimensions. Recently, we turned our attention to gas chromatography on nematic phases as a possible method for the determination of steric differences in PAHs.

Nematic phases are forms of liquid crystals. They are a state of matter intermediate between crystalline solids and "normal" isotropic liquids. They are formed by certain compounds with elongated, relatively polar molecules. Mutual attraction of these molecules tends to orient them with their long axes parallel. Such compounds first melt to an anisotropic liquid, in which the molecules are free to move about only so long as they remain parallel to one another. At higher temperature the melt undergoes a sharp transition to a normal liquid.

It has been observed by several workers[47–49] that on a liquid crystal phase, increased retention correlates (to a first approximation) with increased molecular length-to-breadth ratio of the solute isomers; *e.g.*, *p*-xylene (being more rod-like) can more readily penetrate the liquid crystal phase than *m*-xylene, and thus experience a stronger solute–solvent interaction.

We attempted[50] to correlate the shape parameter (the ratio of the longer to the shorter sides of the rectangular envelope) with chromatographic data for a group of PAHs. We determined the retention indices, I_N, for a series of compounds on the nematic phase BMBT [1.5% of N,N-bis(*p*-methoxybenzylidene)-α,α'-bi-*p*-toluidine on Chromosorb W HP]. We also used the data for I_I obtained by Grimmer and Böhnke[51] for the same compounds on the isotropic phase OV-101. We assumed that interaction forces between the molecules studied and both the nematic and isotropic phases are of the same origin. Thus we could expect the difference in retention indices on the two phases to reflect molecular shape differences. In general, the shape parameter, η, should be described by the equation

$$\eta = k_1 I_N - k_2 I_I + k_3 \tag{8}$$

where k_1–k_3 are constants. We obtained a significant correlation, with a correlation

coefficient of 0.91. Taking into consideration the approximate nature of the shape parameter, η, the correlation is considered to be satisfactory.

4. ELECTRONIC DATA CHROMATOGRAPHICALLY DETERMINED

Following the Hansch approach to QSAR, one is often interested in obtaining numerical data on the electronic properties of the compounds studied. Assuming that polar interactions between the drug and the receptor are of importance for biological effects (which must always be true owing to the hydrophobic nature of the majority of protein receptors[52]), it seems worthwhile finding a convenient method for the determination of molecular polarity. In many instances various kinds of Hammett substituent constants are precise enough, but often additional physicochemical measurements are required. Amongst others, adsorption chromatography could be utilized. However, there are almost no examples of successful QSAR applications of adsorption chromatographic data. Perhaps it is difficult to separate the various interaction forces that take part in this mode of chromatography[53].

In the light of earlier work by Kováts, and especially a recent paper by Gassiot-Matas and Firpo-Pamies[54], it seems possible to obtain some information concerning the electronic character of compounds from their gas chromatographic behaviour. The latter authors[54] suggested that the gas chromatographic retention index is a function composed of two terms: the "bulk" term, related to the solute polarizability, *e.g.*, via its molar refractivity, MR, and thus to the ability to form non-specific dispersion bonds between the solute and stationary phase, and the polar term, related to the solute polarity, *e.g.*, via its dipole moment, μ. Thus the retention index, I, should be generally described as follows:

$$I = a\mu^2 + bMR + c \tag{9}$$

where a, b and c are constants; a is related to the polarity of the stationary phase and b characterizes its polarizability. This idea is in agreement with our earlier observations[55–57] and we have developed it for QSAR purposes[58]. Following the idea for a series of solutes chromatographed on two phases of different polarity and similar molecular weight, the dispersion term should be the same, to a first approximation, and one should obtain

$$I_{NP} = a_1\mu^2 + bMR + c_1 \tag{10}$$

$$I_P = a_2\mu^2 + bMR + c_2 \tag{11}$$

where I_{NP} and I_P are retention indices on non-polar and polar phases, respectively. From eqn. 10 one obtains

$$I_{NP} - bMR = a_1\mu^2 + c_1 \tag{12}$$

Thus, the term $I_{NP} - bMR$ relates directly to a measure of the solute polarity, *e.g.*, μ^2. With the retention indices determined and knowing the value of b, one can easily calculate $I_{NP} - bMR$. The b value can be calculated as follows. From eqns. 10 and 11 one obtains

$$I_P - I_{NP} = (a_2 - a_1)\mu^2 + c_2 - c_1 \tag{13}$$

μ^2 can be obtained from eqn. 10:

$$\mu^2 = \frac{I_{NP} - bMR - c_1}{a_1} \tag{14}$$

Substitution of μ^2 in eqn. 13 by eqn. 14 yields

$$I_P - I_{NP} = \left(\frac{a_2}{a_1} - 1\right)I_{NP} - \left(\frac{a_2}{a_1} - 1\right)bMR - \left(\frac{a_2}{a_1} - 1\right) \cdot c_1 + c_2 - c_1 \tag{15}$$

Then

$$I_P = \frac{a_2}{a_1} \cdot I_{NP} - \left(\frac{a_2}{a_1} - 1\right)bMR - k_3 \tag{16}$$

or

$$I_P = k_1 I_{NP} - k_2 MR - k_3 \tag{17}$$

where k_1–k_3 are constants.

Eqn. 17 is valid and proof has been published. Grzybowski *et al.*[59] published empirical equations relating retention indices on a polar phase NGA, I_{NGA}, and a non-polar phase, SE-30, $I_{SE\text{-}30}$, for a group of 53 phenols via the molecular refractivity, MR:

$$I_{NGA} = 2.12\ (\pm 0.16)I_{SE\text{-}30} - 24.7\ (\pm 2.6)MR + 339.9\ (\pm 182) \tag{18}$$

$n = 32$, $s = 46.1$, $R = 0.9831$;

$$I_{NGA} = 1.94\ (\pm 0.22)I_{SE\text{-}30} - 20.9\ (\pm 6.9)MR + 247.7\ (\pm 243) \tag{19}$$

$n = 11$, $s = 45.9$, $R = 0.9931$; where n is the number of compounds studied, s the standard deviation and R the correlation coefficient.

Thus, having k_1 and k_2 experimentally determined, b can be calculated as follows:

$$b = \frac{k_2}{k_1 - 1} \tag{20}$$

Based on I_{NP}, b and MR (refractivity data are given in ref. 60), the polarity can be determined chromatographically according to eqn. 12.

5. APPLICATIONS IN MEDICINAL CHEMISTRY

Tomlinson[1] reviewed the applications of chromatography in QSAR published before 1975.

Biagi and co-workers were one of the first groups to apply TLC R_M values successfully to the description of various types of biological activity in several groups of chemical compounds. After Tomlinson's publication, Biagi's group reported correlations between R_M values and bioactivity of steroids[61], phenols[62], naphthols and acetophenones[63] and benzodiazepines[64]. With steroids there is a parabolic relationship between the data for haemolytic activity and the membrane binding of the steroids

and their lipophilicity expressed by R_M values. On the other hand, introduction of the R_M^2 term does not improve the correlation between either the protein binding or the duration of action of testosterone esters and lipophilicity. Haemolytic activity, antibacterial activity against *Staphylococcus aureus* and acute toxicity to mice for a group of phenols have been shown to be linearly dependent on R_M values[62]. The data for phenols were later combined with the corresponding data for naphthols and acetophenones[63]. In spite of the fact that the R_M values for phenols, naphthols and acetophenones were determined at different times, the linear relationships between either acute toxicity or haemolytic activity and R_M values for a group of nearly 60 compounds were satisfactory (for five halogenated acetophenones an indicator variable was additionally used). Biagi's group preferred silicone oil as the stationary phase. They found the R_M values from the silicone system to be better correlated with bioactivity of benzodiazepines than those obtained on 1-octanol-impregnated TLC plates.

TLC data obtained with the 1-octanol/acetone–water system served as a better model for the transfer of simple acetanilides from an aqueous environment on to bovine serum albumin macromolecules than did the liquid paraffin/acetone–water system[65]. It was found, however, that the liquid paraffin chromatographic system gave improved replication of the determined ΔR_M values. The logarithm of the intrinsic association constant for the primary binding site was linearly related to ΔR_M.

Linear relationships between TLC partition data and the absorption velocity for a series of 5-substituted barbiturates have been reported by Duran and Plá Delfina[32].

R_M data from the liquid paraffin/acetone–water system gave no significant correlation with antibacterial activity against *Mycobacterium tuberculosis* in a series of isonicotinic acid hydrazide derivatives[66]. Correlation improved when pK_a data were included, thus providing a correction for ionization to the R_M data.

A similar situation has been observed for the tuberculostatic activity of pyrazine carbothioamide derivatives[67] and 2-cyanomethylbenzimidazole derivatives[68]. Here also TLC data from the liquid paraffin–water system gave a satisfactory correlation with bioactivity when used together with spectroscopic data related to polarity.

A parabolic dependence of activity against eight bacterial species on R_M values determined on Kieselgel 60 F_{254} silanisiert plates with acetone–water (60:40) as the mobile phase for a series of α,β-unsaturated γ-lactones was observed by Dal Pozzo *et al.*[69].

Direct partition TLC data on silica gel and cellulose support impregnated with formamide were linearly correlated with the local-surface anaesthetic activity of a series of 2-morpholinoethyl esters of 2-, 3- and 4-alkoxycarbanilic acids[16]. The correlations were derived for *ortho*-, *meta*- and *para*-substituted derivatives separately.

Hulshoff and Perrin[35] obtained an excellent correlation of the R_M values (extrapolated to 100% water in the mobile phase) with the bovine serum albumin binding constants, antihaemolytic activity and the inhibition of Na^+K^+-activated adenosine triphosphatase activity for a series of phenothiazine derivatives. However, the correlations were good only when the biological activities were corrected for the state of ionization. They demonstrated the binding of phenothiazines to albumin to be hydrophobic in origin, which is contrary to the hypothesis[70] suggesting electronic interactions.

TLC partition data proved useful for QSAR studies with a series of rifamycins as inhibitors of viral RNA-directed DNA polymerase and mamalian α- and β-DNA polymerases[71].

Partitioning into erythrocytes of potential antimalarial sulphonamides was also related to R_M values obtained in TLC in the liquid paraffin–phosphate buffer (pH 5) system[72].

Ferguson and Denny[73,74], based on R_M values from partition chromatography, concluded that for the tumour-active but mutagenic 9-anilinoacridines separation of the two classes of bioactivity was possible by simple manipulation of the agent lipophilic–hydrophilic balance.

Chromatographic data have been utilized to evaluate the biological activity of prodrugs. Prodrugs are inactive compounds that undergo metabolic transformation in a living organism into active species. Fujii and co-workers applied R_M values for the description of *in vivo* antistaphylococcal activity in mice of ω-amino acids and their L-histidine dipeptides[12] and carboxylic acids[75].

Maksay *et al.*[76] found a good correlation between pharmacokinetic constants characterizing oxazepam brain levels observed after intravenous administration of produgs (oxazepam esters) and the chromatographic R_M values. They found that an increase in the hydrophobicity of the esters decreased oxazepam brain penetration. To explain this, they suggested that hydrolysis precedes brain penetration and hydrophobicity might primarily influence the hydrolysis rate. The amount of tissue storage, total excretion rates and serum binding was also correlated with hydrophobicity[77].

Another class of pharmacokinetic data (areas under effect–time curves) has been correlated with R_M values for some hypoglycaemic sulphonamides[78].

In spite of the existence of well established[79] HPLC methods for the determination of hydrophobicity, few practical applications in QSAR have been reported. The ability of a short series of 1,3,5-triazine herbicides to inhibit the Hill reaction has been correlated with HPLC retention times determined on ODS columns with water–methanol (95:5) as the mobile phase[80].

An extensive study on the application of HPLC data in correlation with the activity of sulphonamides against *Escherischia coli* and inhibitory potencies of barbiturates on rat brain oxygen uptake and Arbacia egg cell division was described by Henry *et al.*[41].

Data concerning the induction of an enzyme N-demethylase by a group of polycyclic aromatic hydrocarbons have been correlated with the shape parameter calculated from GLC data determined on nematic phases[81]. In the same work, the chromatographic shape parameter was used for a quantitative description of the mutagenicity of the compounds.

If polar interactions between the receptor and drug molecule are of importance for drug action, then the chromatographic measure of polarity[58], determined in a non-polar environment, could better reflect such interactions than Hammett's sigma values, obtained from solution reactions —a drug molecule in contact with the hydrophobic protein of the receptor is actually placed in a non-polar environment. We tried to use the chromatographic measure of polarity for QSAR studies in a group of phenols[58]. We determined the olfactory activity of compounds as detection thresholds. This activity, however, has been satisfactorily described by hydrophobicity parameters and the introduction of no electronic data gives a statistically significant

improvement. On the other hand, the chromatographic polarity measure proved to be useful as a correction factor for ionization to the hydrophobicity parameter.

6. SUMMARY

The rational bases, experimental techniques and conditions required for the chromatographic determination of the structural data of importance for studies on quantitative relationships between chemical structure and biological activity of drugs (QSAR) are reviewed. Practical applications of the information gathered from various chromatographic modes in correlation with bioactivity data are discussed.

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