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Progress in Pharmaceutical Research

Edited by K.R.H. Wooldridge

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Editor's introduction

Pharmaceutical research involves the interplay of various disciplines each with its own highly specialized literature, and the problems facing scientists working in this field to keep themselves well informed are widely recognized. In helping to alleviate this situation, the review article assumes a critical role because it enables the investigator to keep up to date in relevant areas without the necessity of extensive coverage of the primary literature.

In this volume, an effort has been made to present chapters which will provide the expert with the most recent developments, and also give the non-expert a critical survey of new research areas. Obviously it has not been possible to cover progress over the entire field of pharmaceutical chemistry, but topics have been selected which it is hoped will deal in reasonable depth with three different aspects. The chapters cover a widely-applicable technique, the chemistry of an area of drug research which is currently very active, and finally an approach which may well provide the drugs of the future.

In the last few years, the cost in real terms of introducing a new drug to the market has increased very considerably. As a result fewer new products are emerging, and pharmaceutical research organizations are looking to new methods and approaches to make their research more cost-effective.

The immense strides made in computer technology in recent years have had a considerable impact on drug research, and Chapter 1 records recent developments in the increasingly widespread technique of QSAR (Quantitative Structure-Activity Relationships). The technique was originally introduced to relate biological activity and chemical structures within closely related chemical series. In favourable cases a mathematical model can be derived by sophisticated statistical methods which will enable the most active compounds in the series to be identified. In recent years more attention has been paid to the selection of the analogs from which the model is deduced, resulting in more soundly based optimizing procedures.

Perhaps the most exciting possibilities lie in the realms of lead-generation using pattern recognition methods and principal component analysis. Those techniques may lead to quantitative models from which entirely new active structures may emerge. At the present time this remains an intriguing possibility and so far no new drugs have resulted. However, with increasing experience and with the evolution of increasingly sophisticated statistical techniques, there is a very strong possibility that real advances will be made in the next few years.

In 1965 it was discovered that disodium chromoglycate appeared to prevent the release of mediators from mast cells and therefore was able to inhibit antigen-induced bronchospasm when given prophylactically by inhalation. Since then a great deal of work has been carried out to discover other compounds with similar properties, preferably acting orally. The second chapter summarizes the current

state of knowledge in this area and reviews the considerable body of chemistry which has accumulated since 1965.

The final chapter surveys the field of immunostimulants, an area which reflects the changing methods of medicinal research. An immense amount of work has been carried out in the last few years in immunology, but even so the complex mechanisms implied in the subtle control of immunological processes are far from being completely understood. However, immuno-active agents are already widely used in therapy, and the possibility of stimulating the immune response of the host to combat cancer, virus infections, arthritis, etc. are very attractive.

K. R. H. Wooldridge

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Quantitative structure-activity relationships

Keith Bowden and Timothy J. Coombs

1 Introduction

The process which culminates in the identification of a novel medicinal compound is both time-consuming and expensive. With increasing requirements for safety and efficacy, this task promises to become still more challenging. Any means which can be employed to establish a rational approach to drug research may help to offset this trend. The term Quantitative Structure-Activity Relationships (QSAR) embraces one such approach of increasing popularity and successful application, and may be described as a combination of physical organic chemistry, biochemistry and pharmacology using statistical or computational techniques. The objective of QSAR is to investigate and define the interactions between drug molecules and the biological system, whether described by whole animals, single organs, cells or bio-molecules. The spread of interest in QSAR has paralleled the strides made in computer technology in the past twenty years. Several excellent reviews and monographs serve to highlight the rapid evolution of empirical and semi-empirical models.[1–7] The present article seeks to comment upon some of the methods currently popular.

2 Models of quantitative structure-activity relationships in drug design

Quantitative drug design embraces all attempts to relate mathematically biological activity to other properties of molecular structure. This concept was first expressed over a hundred years ago by Crum-Brown and Frazer[8] in the equation

$$\varphi = f(c) \tag{1}$$

where φ is a measure of biological activity and c characterizes the structural features of the drug.

It is clear that in only a limited number of cases can biological activity be successfully related to a single property of molecular structure. Such factors as absorption, distribution and specificity at the site of action contribute towards drug efficacy. Thus the development of models for QSAR has centred upon a rational interpretation of equation (1), which describes the relationships of individual molecular properties to each other and to biological activity.

As the mathematically simplest relationships are linear equations, the majority of QSAR models are essentially attempts to derive an equation of the form shown in equation (2), where x_i are properties of molecular structure, a_o is a constant and a_i describes the contributions of each property to biological activity.

$$\text{biological activity} = a_o + \sum_{i=1}^{m} a_i\, x_i \tag{2}$$

Because of the relatively low precision of biological data, and the uncertainty inherent in applying such models to specific problems, it is necessary to ensure that N, the number of drugs studied, is considerably larger than M, the number properties of molecular structure.

2.1 Multiple regression analysis

The set of coefficients a_i that constitute the solution to equation (2) are usually obtained from the data by the least squares method using multiple regression techniques. Topliss and Costello[9] have pointed out the increasing danger of generating meaningless chance-correlations by this technique, if the number of drugs observed does not surpass the number of variables by a minimum of 5–6 times. Unger and Hansch[10] have also examined the interpretation of regression results for QSAR models, and have described five criteria which must be considered before an optimal equation is identified.

Perhaps one of the most commonly encountered problems associated with the use of regression analysis relates to the collinearity of the variables examined. Hansch has stated[11] that perhaps most published structure-activity studies suffer from this type of poor experimental design. In general, if r is the cross-correlation

term for the regression of one independent variable upon another, r^2 equal to 0.40 is regarded as the maximum acceptable level of chance correlation.

Though an oversimplification, the assumption of a linear additive relationship between biological activity and molecular properties has proven useful in series of structurally related compounds. Few successful QSAR studies have been published [12] involving structurally diverse compounds, although this may relate to the lack of suitable molecular descriptors which can be used to describe diverse structures. In such cases the various methods of pattern recognition may ultimately prove very much more useful.

3 Lead-optimizing techniques

Lead-optimizing techniques are QSAR models in which the principal objective is to improve the biological profile of a lead compound of previously established biological activity. Typically, this could involve increasing the separation between the doses producing desirable and undesirable effects; or the optimization of drug transport to receptor without loss of specificity. This technique is thus limited to the identification of specific molecular properties within a series of compounds associable with an observed biological activity.

The most widely used lead-optimizing techniques are the linear free-energy related Hansch model [13] and the Free-Wilson or additive model. [14]

4 Lead-generating techniques

With a lead-generating technique, the objective is to produce a truly novel therapeutic agent either by utilizing established test systems to search for new lead compounds or by devising new systems or biochemical models.

If it is assumed that the biological activity of a compound is related to the sum of contributions from its component substructures e.g. rings, chains, heteroatoms etc., there is a possibility that quantitative models may be used to develop novel active structures. Each substructural contribution could be assessed from past testing experience and those desirable substructures included in a novel molecule, not containing substructures identified as undesirable. Application of the multiple regression technique is obviously possible. However, the number of substructures generated from chemically unrelated molecules would usually exceed the number of compounds and thus destroy the significance of this approach.

Pattern recognition techniques have been utilized to extract only those substructures which appear critical to biological activity and this approach may thus prove crucial to lead generation.

5 The linear free-energy model

Introduction of different substituents into a lead molecule alters its chemical and,

consequently, its biological properties in a manner which can often be related linearly to the physico-chemical properties of the substituents themselves. A previous knowledge of these physico-chemical properties will thus enable prediction of the activities of numbers of the series as yet to be synthesized. The number of reported investigations devoted to this model, generally associated with the name Hansch, suggest it to be by far the most popular QSAR method.

The term 'linear free-energy relationship' was originally invoked by Hammett.[15] These correlations generally draw a linear relation between the logarithms of the rate coefficients or equilibrium constants of two reactions subjected to the same variations of reactant structure or reaction conditions, equation (3).

$$\log K_A = m . \log K_B + c \tag{3}$$

The existence of such a relationship implies that the reaction variable in question operates upon each reaction series in a similar manner. Hammett[16] successfully applied this concept to correlate the nature of the substituent with reactivity of the side chain of *meta*- and *para*-substituted benzenes, equation (4),

$$\log (k/k_o) = \rho\sigma \tag{4}$$

where k and k_o are the rate coefficients or equilibrium constants of substituted and unsubstituted compounds, respectively. σ is the substituent constant, dependent upon the electronic character and position of the substituent and ρ is the reaction constant, dependent upon the particular reaction and reaction conditions.

Between 1952 and 1966 several workers attempted to apply the Hammett linear free-energy relationship to activity in biological systems. In 1962, Hansen[17] proposed a 'biological Hammett equation', equation (5),

$$-\log (H_\sigma)_q + \log (H_o)_q = \rho\sigma \tag{5}$$

where $(H_\sigma)_q$ and $(H_o)_q$ are the concentrations of substituted and unsubstituted inhibitors of bacterial growth, causing an equivalent biological response. Subsequently, Hansch and Fujita[13] derived an expression which encompassed both σ and the partition constant π, in a single linear free-energy relationship, equation (6),

$$\log \frac{l}{C} = -k\pi^2 + k'\pi + \rho\sigma + k'' \tag{6}$$

where C is the molar concentration of compound required to bring about an equivalent biological response, and k, k' and k'' are constants.

In addition to the original π–σ parameter approximations of hydrophobic and electronic factors involved in drug action, the use of many other free-energy

related physico-chemical parameters has been attempted, e.g. to take into account steric requirements. Some of the most important of the physico-chemical parameters are reviewed individually below.

5.1 *Steric parameters*

Steric requirements of the receptor(s) and drug substance are often of great importance, since both intra- and inter-molecular interactions may be critical. Unfortunately, the description of steric requirements for drug activity using steric parameters remains at a somewhat basic level.

Taft[18] has examined the effect of neighbouring atoms or groups upon a function within the molecule critical for biological activity, by studying a simple physical organic model, i.e. the hydrolysis of aliphatic esters (RCOOR′). Taft postulated that the effect of R on the rate of hydrolysis was due to steric and electronic effects and by separating the latter using the σ^* parameter, the steric parameter E_s could be measured using equation (7).

$$\log (K_R/K_{CH_3}) - \rho\sigma^* = E_s \tag{7}$$

However, the rate of hydrolysis is also said to be affected by hyperconjugation of α-hydrogen atoms in R (α-hydrogen bonding). Hancock *et al.*[19] have proposed a corrected parameter E_s^c which allows for this factor. Despite the restrictive basis of the E_s parameter, it has proved valuable in the interpretation of steric effects in many extremely diverse systems. Unfortunately, only a comparatively small number of experimental E_s values have been determined. However, Charton[20] has shown that E_s values are a linear function of Van der Waal's radii, and Kutter and Hansch[21] have illustrated the application of E_s values calculated from average radii in structure-activity studies.

Fujita and co-workers[22], have examined the E_s and E_s^c values for a set of various alkyl and heteroatom-substituted groups to determine whether they were separable into components in the manner shown in equation (8),

$$E_s(CR_1R_2R_3) = a\,E_s(R_1) + b\,E_s(R_2) + c\,E_s(R_3) + d \tag{8}$$

where $E_s = E_s$ or E_s^c and $E_s(R_1) > E_s(R_2) > E_s(R_3)$. For a set of 37 groups, a very significant correlation for E_s^c values was obtained, 98% of variance in the data being elucidated. This interesting result must at present be viewed as an empirical relationship among steric effect constants for ester reactions; however, its extension to other systems is of considerable significance to QSAR.

Perhaps the greatest limitation to the usefulness of E_s values is that, in common with several other steric parameters, only one aspect of the shape of the substituent is accommodated, in this case, minimum width. This restriction is of

little consequence in the case of spherically symmetrical substituents, e.g. CH_3, CF_3, but many, perhaps most, biologically active molecules possess substituents whose flexibility dictates wide ranges in width and length and whose shape at the receptor is probably determined by several interaction effects. Thus the application of a single steric parameter to the study of nonspherical substituent effects must be viewed with caution, e.g. where steric influence is due to the positioning of a side-chain within a cleft or trough on the receptor, length may be a more relevant parameter than minimum width. Bowden and Young[23] have demonstrated the importance of substituent length (R) in a quantitative structure-activity study with antagonists of acetylcholine and histamine at postganglionic receptors. R is defined as the distance in ångströms from the aromatic carbon atom to which a substituent is bonded, to the periphery of the Van der Waal's radius of the substituent, relative to hydrogen. In recognizing the importance of individual aspects of substituent shape to biological activity, several attempts have been made to incorporate the concepts of width and length in a single parameter. Amoore and co-workers[24] compared the shapes of whole molecules by measuring molecular outlines (silhouettes) in many directions and expressing the results as similarity coefficients. These could then be considered as an average of the differences between molecules in many directions. This approach has been applied to the study of ant alarm pheromone activity of different compounds.[25] Hansch *et al.*[26] have proposed the use of molar refractivity (MR) or molecular weight (MW) as suitable approximations of 'steric bulk'. Although crude, these parameters may be useful measures of 'bulk', and MR in particular, has found application in several published QSAR studies.[27-30] Unfortunately, MR contains an electronic contribution, being directly proportional to the polarizability, and is also often highly collinear with π, especially for apolar groups. Thus its inclusion in multiple regression analysis must be accompanied by cross-correlation analysis of the relevant terms.

Two further parameters are also available for the characterization of 'steric bulk'. The first, based on the topological method developed by Randić[31], has been introduced as the connectivity index χ.[32] This expresses the degree of branching of molecules as a single value which can be correlated with both volume and surface-area related physical properties of a molecule. The relationship of χ with molecular volume is evident in the highly significant correlation with MR, and further studies[32, 34] indicate that other physico-chemical properties, e.g. partition coefficients, E_s, are also highly correlated. Thus the inclusion of χ parameters within the QSAR in order to account for general 'steric bulk' effects, must also be accompanied by a full cross-correlation analysis. The index term χ^v has also been introduced in order to describe molecular connectivity in heteroaromatic systems and is related to χ through the correlation of free valence electrons.

The second, related to the Amoore method discussed previously, is described as the minimal steric difference (MSD).[35] MSD is defined as the non-overlapping volume of the lowest-energy conformations of a given molecule and its 'natural substrate' when orientated to give maximum spatial overlap. Clearly, this method is of limited practical use since the process of molecular superimposition remains subjective and time-consuming, and identification of the 'natural substrate' is seldom a reality. In addition, MSD values must be evaluated for each congeneric series studied and are not therefore generally applicable parameters. There has been considerable investigation of the dependence of steric parameters on the degree of branching in alkyl groups. Bowden and Wooldridge[36] have investigated the application of the Newman 'six number' concept[37] in the evaluation of steric effects caused by 'bulk' interactions which inhibit approach to the reaction centre arising from 'coiled' chains. The Newman system records the number and identity of atoms in a side-chain six atoms distant from a reference atom in the lead skeleton. Useful correlations of bronchodilator activity in a series of substituted 6-thioxanthines were made with partition parameters and/or the steric 'bulk' of side-chain substituents. Significantly, a correlation between the Newman parameters and E_s was apparent, equation (9),

$$E_s = m_1 n_C + m_2 n_H + m_3 \tag{9}$$

where n_C and n_H are the number of carbon and hydrogen atoms in the sixth position respectively.

Charton[38] has also investigated the dependence of the steric parameter ν on the degree of branching in alkyl chains. He defined the parameter by means of the relationship shown in equation (10),

$$\nu_x = r_{vx} - r_{vH} \tag{10}$$

where r_{vx} is the Van der Waal's radius of the substituent x and r_{vH} is that of hydrogen. Unfortunately ν_x values can only be calculated for spherically symmetrical substituents of the type A_3B, e.g. CH_3 or CF_3, or single atom substituents, e.g. Cl or F. These ν values could be correlated with the rates of esterification of substituted carboxylic acids with methanol and ethanol, equation (11).[39]

$$\log k = \psi_\nu + h \tag{11}$$

Steric parameters could thus be obtained for other substituents using this type of reaction, although they can only be applied correctly to a reaction involving a tetrahedral transition state. Charton examined the correlation of all available ν

values for alkyl groups (and subsequently, alkoxy, thioalkyl, dialkylamino and oxyalkyl groups) with substituent branching, equation (12);

$$\nu = an_\alpha + bn_\beta + cn_\gamma + dn_\delta + i \tag{12}$$

where n_α, n_β, n_γ and n_δ represent the number of α, β, γ and δ carbon atoms, respectively. Excellent correlations were obtained by the least mean squares method, indicating that branching accounts for a significant proportion of the variance in steric effects. It is thus unfortunate that the derived regression equations differ for the different types of substituent, restricting the effectiveness of this method for general steric analysis.

Anstel and co-workers[40] have recently reported the formulation of a steric parameter S_b, similar to that of Charton, but more generally applicable, accounting for the nature of the atoms connected to a branching point. The parameter S_b is defined by equation (13),

$$S_b = a_o k_o n_o + a_1 k_1 n_1 + \ldots = \sum_i a_i k_i n_i \tag{13}$$

where n_i indicates the number of non-hydrogen atoms connected to the atoms in position(s) i of a substituent (atom $i = 1$ is not counted). The position i is given by the minimum number of bonds which separate the respective atom from the point of reference (usually the atom of the basic skeleton to which the substituent is linked); itself assigned position zero. The coefficients a_i describe the contribution of branching in position i to the steric effect of the substituent and k_i accounts for the size of the atoms linked to position i. Thus, if there exist several types of non-hydrogen atom linked to position i, S_b is defined by equation (14),

$$S_b = \sum_i \sum_j a_{ij}\ k_{ij}\ n_{ij} \tag{14}$$

where j denotes the type of these atoms. k for carbon (k_C) is arbitrarily chosen to be 1. Values of a_i and k_i have been calculated by interactive regression analysis from E_s and ν values and k-values established for several important atoms (O, S, F, Cl, Br, I) which were generalized to include other elements, i.e. for all 2nd period elements except F ($k_F = 0.8$) $k = 1.0$, and $k = 1.2$, 1.3 and 1.7 for 3rd, 4th and 5th period elements, respectively. Regression analysis has allowed further simplification of equation (14), since the relative contributions towards branching for the positions 0–2 were shown essentially equivalent and those terms in a_i with $i > 3$ could be neglected as in equation (15).

$$S_b = \sum_j k_j\ N_j\ (N_j = \sum_{i=0}^{z} n_{ij}) \tag{15}$$

Although examined in relatively few QSAR to date[40], the S_b parameter compares reasonably well with E_s in a variety of relationships previously shown

sensitive to steric effects. The application of the S_b parameter to every conceivable substituent is a distinct advantage, but further correlation work is required to establish the validity of the model.

Following examination of molecular models of substituents commonly encountered in biological active molecules, Verloop and co-workers[41] have developed the five 'sterimol' parameters, B_1–B_4, which measure widths of substituents in four retangular directions, and L, a measure of the length. These parameters were designed to overcome the problems inherent in many other systems, namely the lack of both general applicability and appreciation of the considerable variation of width in different directions for non-spherical substituents. A computer program has been devised[42] to simulate the construction of a molecular model from which the sterimol parameters are generated. Taking individual atoms in the sequence described by the formula, the program identifies main and branch chains and selects appropriate bond vectors and dihedral angles. From this data, nuclear co-ordinates are generated, together with a connectivity matrix defining the bond structure between atoms. The length and width parameters are derived for those substituent conformations that appear plausible for the free molecules, e.g. from a quantum chemical optimization routine (which minimizes the intramolecular energy) or model building or even intuition. In this respect, the description of flexible substituents by sterimol parameters must be considered approximate, since there is no evidence that the conformation adopted at the receptor is that of the lowest energy stage of the free molecule. However, at the present time it is not practicable to further define the probable conformations at the receptor.

Although the sterimol parameters can, in principle, be calculated for every conceivable substituent, this process requires a computer and associated software. The more detailed description of molecular geometry in terms of width and length inevitably leads to several parameters for a single physico-chemical property, thus demanding a greater number of compounds for meaningful regression analysis. There is also the danger of high intercorrelation between the five steric parameters. The correlation matrix for the 243 substituents so far reported indicated a high probability of intercorrelation between B_2 and B_3 parameters and, to a lesser extent, between B_1 and B_2 and B_1 and B_3. Thus the most prudent approach to QSAR would be to use only the L, B_1 and B_4 parameters. However, it is clear that the sterimol parameters have an important role to play in the examination of steric effects. In several structure-activity relationships in which they are utilized, these parameters give improved correlations over more established parameters[42, 43] and in general produce fewer 'outliers' indicating the acceptability of the model. Clearly, additional important information can also be obtained by correlating specific width or length parameters with activity. Such knowledge could lead to much clearer

interpretation of the directional nature of the steric requirements of the receptor and thus to more active compounds.

In conclusion, it is apparent from both a theoretical and practical standpoint that existing steric parameters remain far from perfect in their application to structure-activity relationships. At present, the best approach towards interpretation of steric effects remains to assess the relative merits and failings of the various parameters in relation to the problem at hand.

5.2 *Hydrophobic parameters*

It was towards the end of the last century that concerted attempts were made to verify the postulate of Crum-Brown and Frazer[8] that the physiological action of a molecule was a function of its chemical structure. Of greatest significance was the separate yet concurrent fundamental studies of Meyer[44] and Overton[45, 46] who discovered the significance of the oil/water partitioning properties of a compound in the expression of its physiological activity. An appreciation of the cellular nature of biological systems has established the necessity for study of such hydrophobic/hydrophilic properties and partition coefficients. It is now quite clear that, even for *in vitro* studies with enzymes or homogenates, the hydrophobic character of interacting molecules may be of great significance. Thus it is not surprising that hydrophobic parameters form an important foundation in the development of the majority of structure-activity relationships.

5.2.1 Measurement of log P

With the recognition of the importance of hydrophobic/hydrophilic character in interaction within biological systems the partition coefficients of biologically active molecules have been measured. The movement of active compounds between aqueous and organic phases is an equilibrium process where the partition coefficient P is the equilibrium constant defined as $P = k_a/k_b$ where k_a and k_b are the rate coefficients for forward and reverse movement between aqueous and organic phases respectively. The value of log P is related to the free-energy changes involved in the movement of active compound between the two phases and may thus be related to biological activity in the linear free-energy equation. The free-energy changes observed for a particular compound will, of course, depend upon the nature of the organic phase and the extent of hydration of the compound.

With the increasing importance of log P values in structure-activity studies it has become desirable to develop a reference solvent system for modelling hydrophobic effects in biological systems. Obviously a system close in physical properties to that of the biological system is desirable. Much empirical testing of

model systems has led to the adoption of the n-octanol/water system as standard.[47] This seems to offer a good compromise with respect to structural, polar and transport properties and n-octanol exhibits hydrogen-bonding acceptor and donor properties typical of many biological macromolecules. Hansch *et al.*[47] have also recorded several further advantages of n-octanol as standard. However, it would appear less acceptable that water should be reference solvent in the aqueous phase. Water is susceptible to quite wide ranges of pH even following double distillation and this effect is particularly marked on storage. With partitioned compounds in which a dissociable acidic or basic group is lacking, the pH of aqueous solvent is of relatively minor consequence, but in the measurement of a dissociable compound (a group which must include a majority of physiologically active structures) a simple buffer system will be of use in the reference system since the degree of dissociation will significantly influence the value of P. The pH value of the buffer would normally be set within the physiological range, 7.2–7.6. However, a carefully selected pH would make it possible to measure P values outside the range practicable with the more usual n-octanol/water system.

In addition to the measurement of P of ionizing compounds at physiological pH, it is often extremely useful to measure the coefficient for both species, since ony one need be the active entity. In most cases, it would normally be sufficient to replace the water or buffer in the aqueous phase with 0.1M hydrochloric acid or 0.1M sodium hydroxide.

Although the majority of recorded partition coefficients[48] have been determined using the traditional method of mixing aqueous and organic phases, this method can be time-consuming and tedious, especially if a series of compounds is studied at a variety of pH values. Reinhardt and Rydberg[49] have developed the 'Akufve' system which enables a rapid and continuous measurement of partition values by incorporating mixing, separation and analysis in a continuous operation.

Various chromatographic techniques have also been investigated and in certain cases, good agreement with the values obtained by n-octanol/water analysis is evident. The use of R_m values obtained by reversed-phase thin-layer chromatography[50, 51] has enabled the determination of partition coefficients using very small quantities of compound. However, the diversity of the solvent systems at present utilized makes comparison of hydrophobicity difficult and the practical range is at present restricted to a few log P units as opposed to the range of 9 or 10 log P units for the traditional n-octanol/water method.

Column chromatography in the form of HPLC has also proved of value in the study of hydrophobicity.[52, 53] Within a series of similar structures, the relative retention times can be related to log P values. This technique offers significant advntages when compounds are impure or in short supply.