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DETERMINATION OF HYDROPHOBICITY PARAMETERS ON POLYBUTADIENE-COATED ALUMINA AND THEIR APPLICATION IN QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIPS ANALYSIS

ROMAN KALISZAN * and JACEK PETRUSEWICZ
Medical Academy, K. Marksa 107, 80-416 Gdansk (Poland)
and
ROGER W. BLAIN and RICHARD A. HARTWICK
Department of Chemistry, Rutgers University, Piscataway, NJ 08854 (U.S.A.)

SUMMARY

The reversed-phase high-performance liquid chromatographic (HPLC) method of hydrophobicity evaluation based on polybutadiene-coated alumina columns was applied in quantitative structure–activity relationship (QSAR) studies of a group of pharmacologically active azole derivatives. The solutes (weak organic bases) were chromatographed at pH 7.30 and 11.5. The HPLC data obtained under alkaline conditions correlated well with hydrophobicity parameter calculated for non-ionized forms of the compounds. The same chromatographic data were demonstrated to provide the most significant information on the biological activity of the compounds under study. The polymer-coated alumina stationary phases developed by Schomburg and co-workers, which can be used over a wide range of eluent pH values and which show no tendency to undergo the specific interactions with solutes typical of silica-based materials, offer unique advantages from the point of view of QSAR applications.

INTRODUCTION

Hydrophobicity (lipophilicity) is a structural feature of compounds that has important effects on biological activity. Determination of the hydrophobicity of the compounds considered is often required in order to rationalize the mechanism of pharmacological action at the molecular level, to predict the activity of drug candidates or other xenobiotics, to evaluate the hazards of environmental pollution, etc.

A single, continuous scale for measurement of hydrophobicity (log P) is provided by the *n*-octanol–water partition system. Numerous limitations of the "shake-flask" method for the determination of log P justified efforts to develop more convenient and reliable chromatographic methods of hydrophobicity characterization[1]. High-performance liquid chromatography (HPLC) is the preferred approach for the determination of lipophilicity, having the following advantages: it is fast and suitable for substances containing impurities, for mixtures and for volatile compounds; it

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requires no quantitative determination; it is highly reproducible and can be applied over a wide hydrophobicity range; and it provides precise control of pH and ionic strength during the separation process.

The reversed-phase HPLC systems most often used for hydrophobicity characterization employ alkyl ligands chemically bonded to the silica support surface[1–4]. There are two main disadvantages of the hydrocarbonaceous silica stationary phases: the possibility of interaction of some solutes with the free silanol sites on the silica support surface and the chemical instability of alkyl-bonded silicas at pH above *ca.* 8 (ref. 5).

In a search for a stationary phase allowing hydrophobicity comparisons of undissociated organic bases, we considered the new generation of polymer-coated reversed-phase stationary phases introduced by Schomburg and co-workers[6,7]. Using such a stationary phase, we proposed[5] an HPLC method for the determination of the hydrophobicity of diverse basic, neutral and acidic solutes of different chemical classes. The capacity factors (log k') determined for a set of 22 test solutes were linearly correlated with the *n*-octanol–water partition coefficients (log P).

In this paper we report the results of studies on the application of the previously proposed method of hydrophobicity evaluation to the determination of quantitative structure–activity relationships (QSAR). The compounds of interest are newly synthesized azole-type organic bases possessing varying circulatory activity[8]. The biological activity data, pIC_{30}, determined independently[9,10], are the negative logarithms of the molar concentrations of the compounds giving 30% inhibition of the human blood platelet aggregation caused by 10^{-5} M adrenaline.

The lipophilicity of the compounds in a non-ionized form, which is assumed to interact with biological receptors, is of interest for QSAR studies. On the other hand, the overall lipophilicity of the non-ionized and ionized species at physiological pH may also be important, as it may be related to the penetration of drug in living systems, and thus to its availability to specific receptors located in various body compartments. With this in mind, we planned our HPLC determinations at pH 7.30 and 11.5. For the sake of comparison the hydrophobicity, Σf, of the agents studied was calculated on the basis of the so-called fragment method of Hansch and Leo[11]. The HPLC data were related to the hydrophobicity data derived from the structural formulae of the agents studied and also to their biological activity data.

EXPERIMENTAL

Chromatography

The chromatographic system (Altex Scientific, Berkeley, CA, U.S.A.) consisted of a single-piston reciprocating pump and a Model 157 UV detector operating at 254 nm. A Rheodyne (Cotati, CA, U.S.A.) Model 7410 injection valve fitted with a 10-μl sample loop was used. A 150 × 4.6 mm I.D. stainless-steel column was slurry-packed using 2-propanol as the slurry solvent and methanol as the packing solvent. Polybutadiene-coated Spherisorb A5Y stationary phase was prepared according to the procedure of Schomburg and co-workers[6,7]. The polybutadiene was immobilized on the alumina support surface with the help of cross-linking reactions involving radical formation.

As a compromise between reasonable retention times for the most strongly

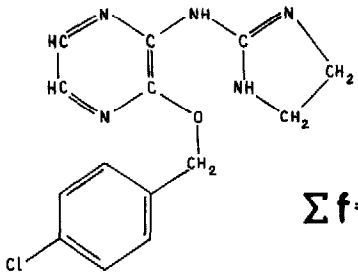
retained solutes and a reliable pH for the control of ionization, a methanol–buffer (1:1, v/v) eluent was used. Two buffers were applied: for pH 7.30 the buffer was 0.1 *M* potassium dihydrogenphosphate–0.1 *M* sodium hydroxide and for pH 11.5 the buffer was 0.05 *M* disodium hydrogenphosphate–0.1 *M* sodium hydroxide.

The structures of the solutes under study are given in Table I; a general procedure for their preparation is described elsewhere[8].

To calculate the capacity factors, k', the solvent disturbance peak was used as a reference. Reproducible data for the solvent disturbance peak were obtained when trace amounts of methanol were injected into the column. The logarithms of the capacity factors, $\log k'$, given in Table I are the means of three determinations.

Structural analysis

The method of calculation of the hydrophobicity parameter, Σf, according to Hansch and Leo[11] is illustrated in Fig. 1. The superscript φ denotes substitution in aromatic systems; fragments fused in an aromatic ring are underlined; subscript b is a geometric factor proportional to the length minus 1 of a chain (no superscript) or a ring (superscript ring); the subscript (=) represents unsaturation; F_{HBN} is a correction



$\Sigma f = 2.52$

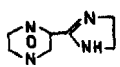
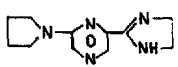
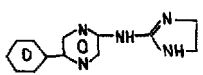
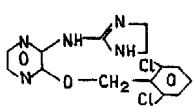
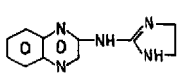
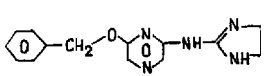
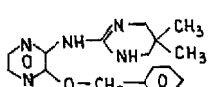
f_{Cl}^{φ}	= 0.94
$f_{C_6H_4}$	= 1.67
f_{CH_2}	= 0.66
f_{O}^{φ}	= 0.61
$F_b \times (3-1)$	= −0.24
$f_{\underline{N}} \times 2$	= −2.24
$f_{\underline{CH}} \times 2$	= 0.70
$f_{\underline{C}} \times 2$	= 0.26
f_{NH}^{φ}	= −1.03
f_{C}	= 0.20
f_{NH}	= −2.15
f_{N}	= −2.38
$f_{CH_2} \times 2$	= 1.32
$F_b \times (2-1)$	= −0.12
$F_b^{ring} \times (5-1)$	= −0.36

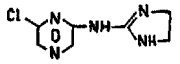
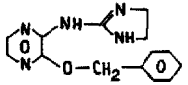
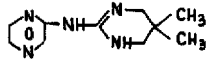
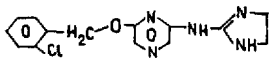
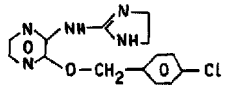
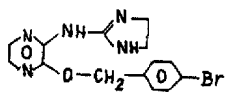
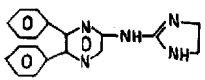
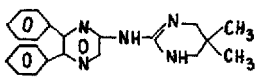
$F_{(=)}$	= −0.55
F_{HBN}	= 0.60
F_{P2}^{φ}	= −0.08(−1.12 −1.12) = 0.18
F_{P2}^{φ}	= −0.08(−1.12 −0.61) = 0.14
F_{P1}^{φ}	= −0.16(−1.12 −0.61) = 0.28
F_{P2}^{φ}	= −0.08(−1.03 −0.61) = 0.13
F_{P2}^{φ}	= −0.08(−1.12 −1.03) = 0.17
F_{P1}^{φ}	= −0.16(−1.12 −1.03) = 0.34
F_{P2}^{φ}	= −0.08(−1.12 −0.61) = 0.14
F_{P1}^{ring}	= −0.32(−2.38 −1.03) = 1.09
F_{P1}^{ring}	= −0.32(−2.15 −1.03) = 1.02
F_{P1}^{ring}	= −0.32(−2.38 −2.15) = 1.45
F_{P2}^{ring}	= −0.20(−2.38 −2.15) = 0.91

Fig. 1. Sample calculations of the hydrophobicity parameter, Σf, by the fragment method of Hansch and Leo[11]. For details, see text.

TABLE I

STRUCTURES, CAPACITY FACTORS, HYDROPHOBICITY PARAMETERS AND ANTI-AGGREGATORY ACTIVITIES OF PYRAZINYLDIAZACYCLOALKANES

No.	*Structure*	*Log k'*		*Σf*	*pIC*$_{30}$
		pH 11.5	*pH 7.30*		
1′		−0.353	−0.125	−1.41	9.68*
2′		0.160	−0.085	0.49	10.72
3′		0.708	0.434	0.71	10.22
4′		1.163	0.836	3.23	8.80
5′		0.054	0.091	0.61	8.13**
6′		0.582	0.398	0.70	10.20
7′		1.050	0.776	2.55	9.96

8′		−0.109	−0.262	−0.25	9.68*
9′		0.755	0.410	1.81	9.33
10′		0.301	0.015	0.11	10.22
11′		0.831	0.702	1.41	9.12
12′		1.270	0.760	2.52	8.11
13′		1.402	0.879	2.67	—***
14′		1.145	0.905	2,14	8.66
15′		1.913	1.279	3.45	—***

* Partial agonist.
** Full agonist.
*** No 30% antagonist effect attained.

for intramolecular hydrogen bonding; and F_P factors account for the H-polar proximity effects when two H-polar fragments are separated by one (subscript 1) or two (subscript 2) carbon atoms. The sum of individual fragments and correction factors yields the total hydrophobicity, Σf (Table I).

Pharmacological data

Anti-aggregatory activity of the compounds studied was determined independently[9,10] by the turbidimetric method of Born[12]. Of the fifteen compounds studied (Table I), compound 5′ showed significant aggregatory activity. The anti-aggregatory effects of compounds 13′ and 15′ were less than 30% at the highest concentrations obtained. The anti-aggregatory activity data, pIC_{30}, are given in Table I.

RESULTS AND DISCUSSION

The correlation between capacity factors determined at pH 7.30 and 11.5 is shown in Fig. 2. Deviation of the data points from a straight line results from differences in the degree of ionization of the solutes at the two pH values studied. The compounds studied are weak bases, but at pH 7.30 at least some of them are still partially ionized. As pointed out by the referee, the log k' values decreased for compounds 1′ and 5′ when measured at higher pH values. This is opposite to the expected behaviour and may indicate interaction between the solute and the alumina backbone.

Figs. 3 and 4 shows relationships between Σf and log k', as determined at pH 7.30 and 11.5, respectively. Evidently, the correlation between Σf and log k' determined at pH 11.5 is better. For the total number of solutes (n = 15) the correlation coefficient, r, is 0.944, the standard deviation from regression, s, is 0.223 and the

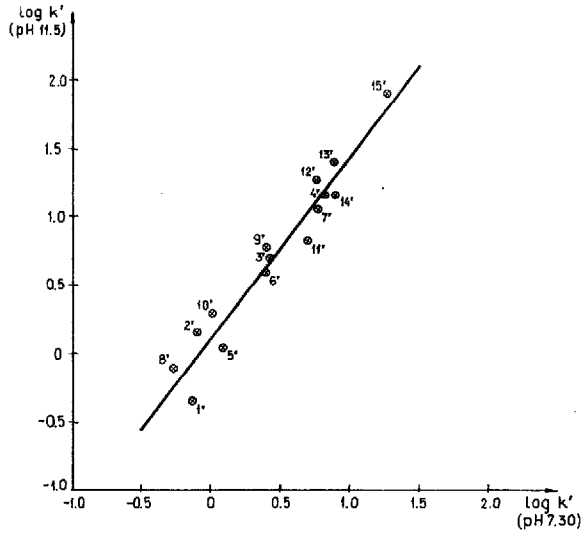


Fig. 2. Correlation between the HPLC capacity factors determined at different pH values for the solutes listed in Table I. log k' (pH 11.5) = 1.323 log k' (pH 7.30) + 0.106 (n = 15, r = 0.969, s = 0.168, p = 5.4 · 10^{-8}).

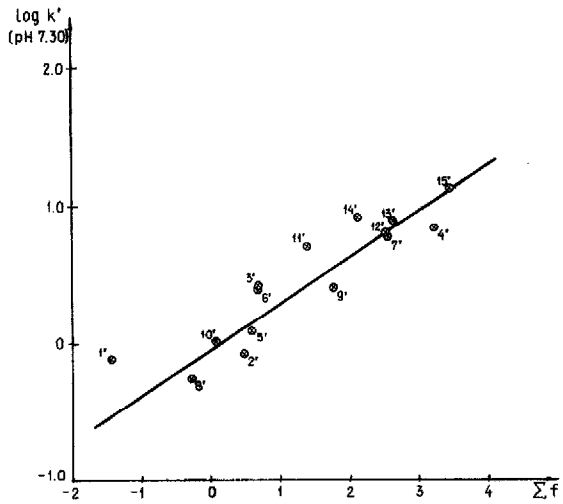


Fig. 3. Relationship between the theoretically calculated hydrophobicity parameter, Σf, and the capacity factor, log k', determined at pH 7.30. Compounds are numbered as in Table I. log k' (pH 7.30) = 0.301 Σf + 0.052 (n = 15, r = 0.915, s = 0.199, $p = 1.8 \cdot 10^{-5}$).

significance level, p, is $1.7 \cdot 102^{-6}$. The respective data for the relationship Σf *vs.* log k' (pH 7.30) are r = 0.915, s = 0.199 and $p = 1.8 \cdot 10^{-5}$. As the Σf values represent hydrophobicities of non-ionized forms of the compounds, the log k' data for bases determined at higher pH shoud correlate better with Σf, as occurs here.

Fig. 5 shows the relationship between Σf and bioactivity data, pIC_{30}. Although the scattering of the data is obvious, some regularities are apparent. Keeping in mind

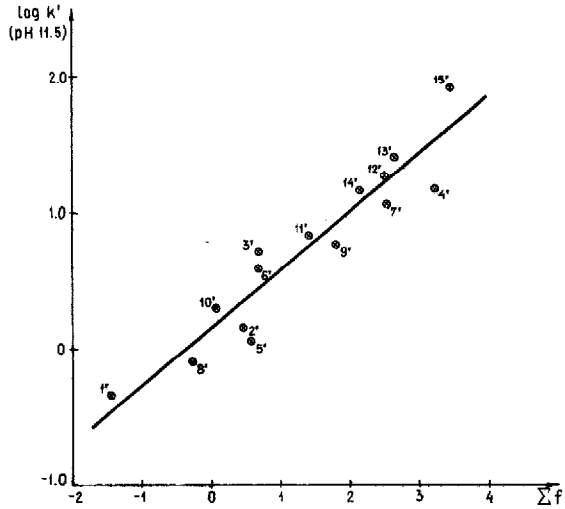


Fig. 4. Relationship between the theoretically calculated hydrophobicity parameter, Σf, and the capacity factor, log k', determined at pH 11.5. Compounds are numbered as in Table I. Log k' (pH 11.5) = 0.424 Σf + 0.139 (n = 15, r = 0.944, s = 0.223, $p = 1.7 \cdot 10^{-6}$).

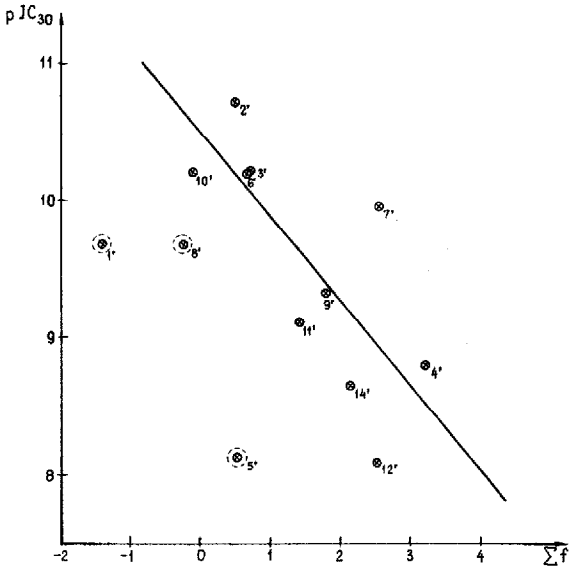


Fig. 5. Anti-aggregatory activity, pIC_{30}, as a function of the theoretically calculated hydrophobicity parameter, Σf. Compounds are numbered as in Table I. Compounds 1', 5' and 8' were excluded from the regression analysis. $pIC_{30} = -0.62\ \Sigma f + 10.50$ ($n = 10$, $s = 0.63$, $r = 0.761$, $p = 4.8 \cdot 10^{-2}$).

the generally limited precision of the determination of hydrophobicity by means of the hydrophobicity parameter, Σf, one may conclude that the anti-aggregatory activity of the compounds studied decreases with increasing hydrophobicity.

A similar trend is observed in Fig. 6, where the same biological activity data are

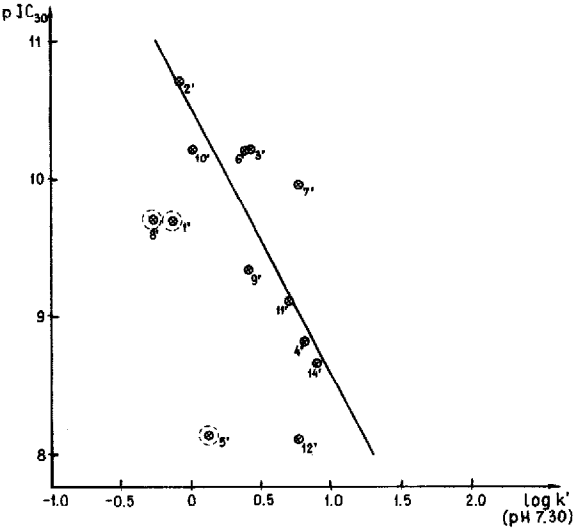


Fig. 6. Anti-aggregatory activity, pIC_{30}, as a function of the HPLC capacity factors determined at pH 7.30. Compounds are numbered as in Table I. Compounds 1', 5' and 8' were excluded from the regression analysis, $pIC_{30} = -1.94 \log k'$ (pH 7.30) + 10.53 ($n = 10$, $s = 0.60$, $r = 0.784$, $p = 3.5 \cdot 10^{-2}$).

plotted against log k' values determined at pH 7.30. The scattering of the data points in Fig. 6 is less than that in Fig. 5, but the predictive value of the pIC_{30} *vs.* log k' (pH 7.30) relationship is not better than the pIC_{30}*vs.* Σf relationship.

The correlation between pIC_{30} and log k' (pH 11.5) is shown in Fig. 7. The observed relationship is satisfactory from the point of view of biological QSAR, especially if one realizes that hydrophobicity is an important but by no means exclusive structural factor affecting biological activity. The obvious outlier is compound 5', which was proved to be an agonist. Compounds 1' and 8' are also outliers. The possible explanation for this is that these compounds possess mixed aggregatory and anti-aggregatory properties. Such a phenomenon was observed independently in pharmacological studies of a group of structurally related imidazoline drugs[13].

Compounds 13' and 15' require separate discussion. For these highly hydrophobic solutes no 30% inhibition of the adrenaline-induced aggregation was obtained at concentrations of up to 10^{-8} *M*. However, if one extrapolates the plot of percentage inhibition against the concentration of the compound to 30% inhibition of aggregation, then the extrapolated biological activity values, pIC_{30} (extrapolated), fit the relationship shown in Fig. 7.

In QSAR studies, the physical interpretation of the relationships is important. The results obtained here clearly indicate that the more hydrophobic the compound, the lower is its ability to produce 30% inhibition of the adrenaline-induced aggregation. Such an observation could appear surprising, as the reverse dependence of biological activity on hydrophobicity is more commonly reported. However, a similar hydrophobicity–biological activity dependence was found by Hansch and Steward[14] as early as 1964. They linked the concentration of penicillin derivatives needed to cure mice of a bacterial infection to the hydrophobicity of the substituents. In subsequent

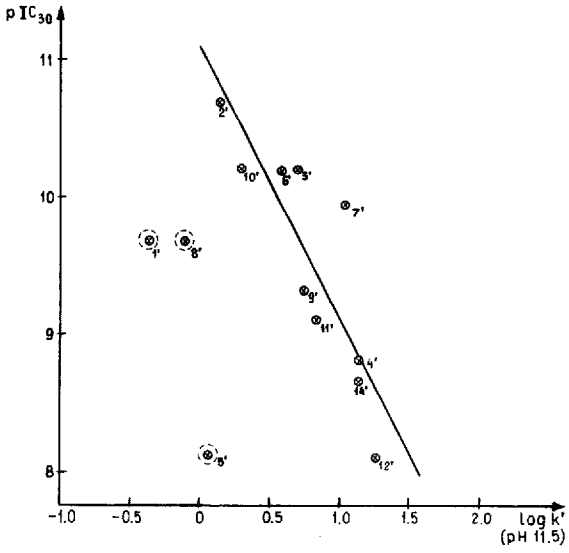


Fig. 7. Anti-aggregatory activity, pIC_{30}, as a function of the HPLC capacity factors determined at pH 11.5. Compounds are numbered as in Table I. Compounds 1', 5' and 8' were excluded from the regression analysis. pIC_{30} = −1.95 log k' (pH 11.5) + 11.09 (n = 10, s = 0.50, r = 0.856, p = $1.0 \cdot 10^{-3}$).

studies it was demonstrated that the observed hydrophobicity–biological activity relationship for penicillins resulted from the fact that hydrophobicity promoted binding to a different than the pharmacological receptor site of loss to a greater extent than it promoted antibacterial activity[15]. Similarly, the binding to human serum albumin can also reduce the effective concentration of the compounds studied here, reaching the target, *i.e.*, blood platelet adrenoceptor.

From the chromatographic point of view the most important conclusion is that the HPLC method for the evaluation of hydrophobicity employing polybutadiene-coated alumina columns readily provides structural information directly applicable to QSAR studies. The chemical stability of the new polymer-coated reversed-phase materials over a wide range of eluent pH values, including alkaline environments, is especially valuable. In effect, the hydrophobicity of non-ionized forms of solutes, including organic bases, can be directly quantified by HPLC.

Leaving aside the question of what the hydrophobicity really is, we realize that individual hydrophobicity parameters depend on the method of determination. Also, the semi-empirical fragmental methods for the evaluation of hydrophobicity do not yield absolute quantities because of some ambiguities in fragment and/or correction factor definition and identification. Hence the method of choice should be one that produces the best QSAR. In this work, the HPLC method for the determination of the hydrophobicity of basic solutes at pH 11.5, employing polybutadiene-coated alumina columns, yielded better QSAR than the theoretically calculated hydrophobicity parameter or HPLC data obtained at the pH at which ordinary reversed-phase hydrocarbonaceous silica columns can be operated.

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