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Hydration of substituted benzenes. Experimental studies and relationship with lipophilicity

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Lipophilicity is a molecular property describing the distribution of compounds between two non-miscible phases and playing a major role in drug design and in the study of structure–activity relationships (SAR). An impressively large number of publications report relationships between lipophilicity and a variety of biological activities, and one can only be intrigued by the power and success of lipophilic parameters in SAR. One of the causes of this effectiveness can be ascribed to the fact that partition processes dominate many interactions between xenobiotics and biosystems, but what additionally makes lipophilicity so popular an object of scientific enquiry is its informativeness in terms of fundamental physicochemical and structural properties.

In an extensive review article (Van de Waterbeemd and Testa, 1987), some of us have examined the structural information content of lipophilicity, concluding that it encodes two major contributions which are a volume (or cavity) term, and polarity terms. Thus, the partition coefficient of a large number of solutes has been factorized into volume, dipolarity, and hydrogen-bond acceptor basicity (Taft et al., 1985). The polarity factors in lipophilicity express various solute–solvent interactions of an electrostatic and directional nature, and as such they account for a number of electrostatic interactions between xenobiotics and biological binding sites (receptors, enzymes, ...).

A few years ago, a role has been postulated for hydration factors in lipophilicity (Van de Waterbeemd and Testa, 1983). Because hydration and dehydration processes are of importance in the thermodynamics of ligand binding (e.g. Testa et al., 1987), the interpretation of lipophilicity values in terms of solute hydration might, when within reach, contribute to a better understanding of structure–affinity relationships. A number of physical, electrochemical and spectroscopic methods exist to experimentally investigate water–solute interactions and to determine hydration energies (e.g. Amis and Hinton, 1973), but to the best of our knowledge no experimental study has examined possible relationships between lipophilicity and hydration in series of solutes. The present work is an explorative study in this direction. Fifteen substituted benzene derivatives were used, the lipophilicity of which had been previously measured by a reversed-phase HPLC method (Tsantili-Kakoulidou et al., 1987). These compounds were partitioned between water and a

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highly apolar solvent, namely carbon tetrachloride, and hydration was measured as the amount of water dragged into CCl_4 by the solute (i.e. water concentration in the CCl_4 solution minus water concentration in the blank CCl_4). Two methods were used to titrate the water content of CCl_4, namely ^{1}H-NMR spectroscopy and Karl–Fischer titrimetry.

The solute (0.005 mol = 1.0 M) was dissolved in 5.0 ml of freshly redistilled CCl_4, and the solution shaken with 5.0 ml of distilled water for 10 min. The organic layer was separated after decanting and centrifugation. The fraction of solute lost into the aqueous phase was measured by a RP-HPLC method and corrected for (range 0.03–0.9% depending on the solute). When the water in CCl_4 was titrated by ^{1}H-NMR (Varian VXR-200 spectrometer), toluene (0.01 M) was added as internal standard, the protons of H_2O and of the methyl group in toluene resonating at 1.47 and 2.56 ppm, respectively. A Metrohm 652 KF coulometer was used to titrate water by the Karl–Fischer method. For each determination, 3 solutions and 3 blanks were prepared, and the differences between solutions and blanks afforded the amount of water dragged into the organic solvent by the solute. This amount was expressed as the number of molecules of water per 1000 molecules of solute.

In a first experiment, it was verified that the amount of water dragged into CCl_4 was proportional to solute concentration. Using nitrobenzene at 6 concentrations ranging from 0.25 to 1.50 M and titrating water dissolved into the organic phase by ^{1}H-NMR, a good proportionality was indeed found (Table 1) ($n = 7$; $r = 0.985$).

TABLE 1

Effect of nitrobenzene concentration on the total concentration of water dissolved in CCl_4 (^{1}H-NMR titration)

Nitrobenzene concentration (M)	Total water concentration (values in mM ± S.D.)
0	5.3 ± 0.4
0.25	5.8 ± 0.2
0.50	9.5 ± 1.2
0.75	10.4 ± 0.2
1.0	12.8 ± 0.6
1.25	13.4 ± 0.6
1.5	16.2 ± 1.0

TABLE 2

Hydration, Van der Waals volumes and lipophilicity of benzene derivatives

Benzene derivative	HYD_{NMR} [a]	HYD_{KF} [b]	VOL [c]	log k_w [d]
-chloro	1.0 ± 1.1	2.4 ± 0.4	64.12	2.849
-methoxy	10.5 ± 0.8	7.9 ± 0.3	71.56	2.094
-nitro	6.5 ± 0.7	8.7 ± 0.4	68.88	1.917
-dimethoxy				
ortho	19.8 ± 0.7	22.0 ± 0.3	90.08	1.741
meta	4.1 ± 0.8	10.2 ± 0.5	90.08	2.347
para	5.2 ± 1.0	10.9 ± 1.0	90.08	2.144
-methoxynitro				
ortho	12.6 ± 0.5	17.2 ± 0.6	87.40	1.896
meta	7.3 ± 0.7	12.2 ± 0.6	87.40	2.431
para	11.0 ± 0.2	12.4 ± 0.5	87.40	2.312
-chloromethoxy				
ortho	6.5 ± 0.6	5.9 ± 0.4	82.64	2.608
meta	1.7 ± 0.4	7.7 ± 0.7	82.64	3.052
para	3.6 ± 0.2	6.8 ± 0.4	82.64	2.979
-chloronitro				
ortho	5.5 ± 0.9	7.8 ± 0.3	79.96	2.517
meta	4.1 ± 0.5	7.5 ± 0.4	79.96	2.616
para	7.1 ± 1.4	7.9 ± 0.5	79.96	2.448

[a] Number of water molecules per 1000 molecules of solute (± S.D.), measured by ^{1}H-NMR as explained in the text.
[b] Same as (a), but measured by Karl–Fischer titration.
[c] Van der Waals volumes in cm^3/mol, calculated according to Testa and Seiler (1981).
[d] Lipophilicity values taken from Tsantili-Kakoulidou et al. (1987).

The results for the 15 solutes are reported in Table 2, showing some marked discrepancies between the two titration methods ($n = 15$; $r = 0.865$). Similar discrepancies were also noted when water concentrations were measured in the blanks (detailed data not shown). With the ^{1}H-NMR method, the water concentration was found to be 4.7 ± 1.3 mM ($n = 45$), while the Karl-Fischer method yielded 7.3 ± 0.3 mM ($n = 45$). Thus the ^{1}H-NMR method appears less precise than the Karl–Fischer method (S.D. 28% vs 4%), and the discussion to follow will be restricted to results obtained by the latter method.

The relationship between hydration and lipophilicity is displayed in Fig. 1 and corresponds for

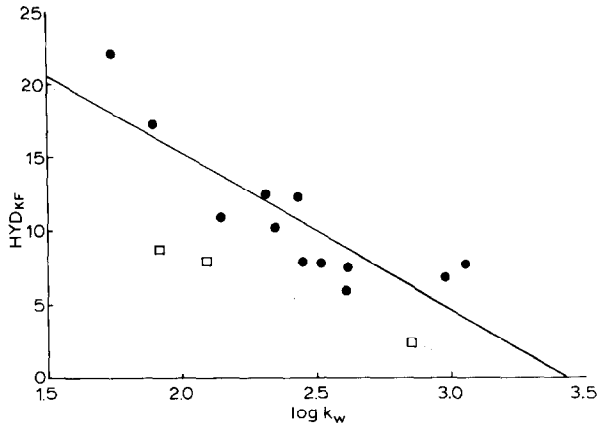

Fig. 1. Plot of lipophilicity (log k_w values) vs hydration of 3 monosubstituted (□) and 12 disubstituted benzenes (●).

all 15 solutes to Eqn. 1 (95% confidence limits in parentheses):

$$\log k_w = -0.060(\pm 0.034)\mathrm{HYD}_{KF} + 2.99(\pm 0.37)$$

$$n = 15;\ r = 0.731;\ s = 0.278 \qquad (1)$$

As suggested by Fig. 1, a better correlation exists when the 12 disubstituted benzenes are considered separately:

$$\log K_w = -0.069(\pm 0.029)\mathrm{HYD}_{KF} + 3.16(\pm 0.34)$$

$$n = 12;\ r = 0.857;\ s = 0.208 \qquad (2)$$

When the 3 monosubstituted benzenes are considered alone, a good correlation exists between log k_w and HYD_{KF} ($n = 3$; $r = 0.998$), and the same is true for the 4 ortho-disubstituted benzenes ($n = 4$; $r = 0.993$). The same is not verified for the meta and para derivatives. Such a difference may be related to the existence of a stronger dipole moment in the monosubstituted and ortho-disubstituted derivatives, but a larger number of solutes will have to be investigated before attempting any rationalization.

To investigate whether the present data could lead to a factorization of lipophilicity into hydration and volume terms, the Van der Waals volume of the compounds was calculated and entered into Eqn. 1 to yield Eqn. 3:

$$\log k_w = 0.468(\pm 0.489)\mathrm{VOL} - 1.05(\pm 0.49)\mathrm{HYD}_{KF}$$

$$n = 15;\ r = 0.815;\ s = 0.246 \qquad (3)$$

Note that Eqn. 3 is presented in normalized form the regression coefficients of which assess the relative contributions of the independent variables (Mager and Barth, 1979). The VOL variable in Eqn. 3 is marginally significant and as compared to Eqn. 1 improves only moderately the quality of the statistics, presumably due to the limited variation in VOL values (see Table 2). Nevertheless, Eqn. 3 is viewed as promising in that it suggests that the HYD_{KF} parameter can indeed serve as an *experimentally* determined polarity descriptor in factorizing lipophilicity.

In conclusion, the present study explores two experimental techniques to quantitate the affinity of solutes for water in an apolar environment. Karl–Fischer titrimetry proved to be faster, more sensitive and markedly more precise than ^{1}H-NMR. One limitation of the technique described is that polar solutes could not be investigated due to their lack of solubility in CCl_4. The choice of this solvent was imposed by the NMR technique, but other non-polar solvents can be chosen when Karl–Fischer titrimetry is used alone. These solvents should dissolve rather polar solutes but must not form electrostatic interactions with water. Toluene is such a solvent (data not shown). Other improvements are necessary (e.g. increased sensitivity to lessen solute concentration) before this approach can be exploited, and theoretical studies (e.g. Monte Carlo and quantum mechanical calculations) should be performed to interpret the results in physicochemical terms. Nevertheless, the present study suggests that experimentally determined hydration factors may prove fruitful in drug design.

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