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Hydrophobicity parameter from high-performance liquid chromatography on an immobilized artificial membrane column and its relationship to bioactivity

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Abstract

There are numerous measures of hydrophobicity employed in the quantitative structure–bioactivity relationship studies. Individual bioactivity parameters may appear best predicted by the specific hydrophobicity parameters. Introduction of an original reversed-phase material, the immobilized artificial membranes (IAMs) opened new perspectives for bioactivity predictions in the case of the hydrophobicity-driven processes. Comparative studies of performance of the HPLC-derived hydrophobicity measures demonstrate the advantages of the IAM-derived hydrophobicity parameters in predicting the human skin permeation by non-ionizable agents as exemplified by steroid hormones. On the other hand, the skin permeation data of agents ionized at physiological pH appeared less dependent of the retention on IAMs. The IAM columns increase the means of characterization of various aspects of hydrophobicity. Their advantages over the slow equilibrium methods of hydrophobicity determination are the simplicity of operation and the suitability of the generated retention measures for predicting specific bioactivity parameters.

1. Introduction

The *n*-octanol–water liquid–liquid partition system is the common reference system providing the most popular scale of hydrophobicity, i.e., logarithm of partition coefficient, log P [1]. Although the n-octanol–water system cannot be assumed the best possible model for all biological permeation barriers and receptor binding sites, large compilations of log P data [2] form the basis for many satisfactory bioactivity predictions. On the other hand, tediousness and poor reproducibility of hydrophobicity determination by the slow equilibration methods called the attention of researchers towards reversed-phase high-performance liquid chromatography (RP-HPLC) as a tool for a convenient assessment of relative hydrophobicities within a series of solutes.

The RP-HPLC systems commonly applied for hydrophobicity determination employ octanol-like or hydrocarbonaceous stationary phase materials [3]. Recently, a new RP-HPLC stationary phase material became available which models natural membranes, namely the so-called immobilized artificial membrane (IAM) [4]. The IAM phases form cofluent monolayers of immobilized membrane lipids (lecithin), wherein each lipid molecule is covalently bound to propylamine-silica. Unreacted propylamine moieties

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can additionally be end-capped with methylglycolate. Membrane lipids possess polar headgroups and two non-polar chains. One of the alkyl chains is linked to the propylamine-silica surface. The immobilized lipid headgroups protrude away from the stationary phase surface and are the first contact site between solutes and IAM [5].

Preliminary studies have demonstrated a good correlation between retention data determined on IAMs and human skin permeation for short series of alcohols and steroids. The same biological data showed poor correlation with chromatographic retention parameters determined on a regular hydrocarbonaceous reversed-phase column [4,5]. Kaliszan et al. [6] demonstrated weak correlations ($R = 0.8$) between retention data determined on the IAM-type columns and the reference hydrophobicity measure, log P, for a series of psychotropic and antihistamine drugs. There was also a very weak correlation between the logarithm of the capacity factor from the IAM column, log k'_{IAM}, and the extrapolated to pure buffer eluent logarithm of capacity factor determined on a deactivated hydrocarbonaceous silica column [6]. Recently, Kaliszan et al. [7] reported high correlation between log k'_{IAM} determined with buffers of physiological pH and the ionization-corrected reference hydrophobicity parameter from the n-octanol–water system for a group of β-adrenolytics ($R = 0.962$) and a poorer correlation ($R = 0.839$) for a series of phenothiazines. Regression analysis of several pharmacokinetic and other bioactivity data for the agents under study in terms of hydrophobicity parameters demonstrated the performance of log k'_{IAM} to be as good as that of the reference hydrophobicity parameters in predicting of the activity of drugs within biophase.

In this report we further confirm the observation that retention data determined in a simple, fast and reproducible manner on IAM columns comprise information on properties of series of xenobiotics which are distinctive from those provided by the n-octanol–water systems. We compare the performance of different hydrophobicity scales in predicting a human skin permeability.

2. Experimental

2.1. Materials

Three test series of the compounds were analyzed: steroid hormones, phenolic derivatives and a group of agents known to permeate skin in an ionized form.

The following steroids were chromatographed: estradiol, estriol, estrone, hydrocortisone, pregnenolone, progesterone, testosterone (all from Sigma, St. Louis, MO, USA), aldosterone, cortisone and deoxycorticosterone (all from Koch-Light Labs., Colnbrook, UK).

The test group of phenolic compounds was made up of the following derivatives: p-bromophenol, chlorocresol, p-chlorophenol, o-cresol, m-cresol, p-cresol, p-ethylphenol, m-nitrophenol, p-nitrophenol (all from Aldrich, Steinheim, Germany), methylhydroxybenzoate (Unia Labs., Warsaw, Poland), β-naphthol, phenol, resorcinol, thymol (POCh, Gliwice, Poland).

The group of solutes ionizable at physiological conditions consisted of: baclofen (Polfa, Starogard, Poland), L-phenylalanine, L-tyrosine, L-tryptophan (all from Sigma), sodium salicylate (Polfa).

2.2. Human skin permeation data

Logarithms of permeability coefficient, log K_p, had been chosen as measures of skin permeability by various solutes.

The human skin permeation data (log K_p) of steroid hormones were taken from Scheuplein et al. [8]. The permeability data of phenolic compounds across human epidermis were taken from Roberts et al. [9]. The respective skin permeation data for the test series of ionizable agents were found in Ruland and Kreuter [10], Mazenga et al. [11] and Kurihara-Bergstrom et al. [12].

2.3. Reference partition coefficients

The human skin–water partition coefficient data (log P_m) for a group of steroid hormones

were found in literature [8]. Reference *n*-octanol–water partition coefficients for the three series of test compounds were taken from Refs. [2], [13] and [14].

2.4. Determination of hydrophobicity by HPLC on an IAM column

The column used was formed by 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-*sn*-3-glycerophosphocholine (lecithin-COOH) bonded to silica-propylamine with the unreacted propylamine moieties end-capped with methylglycolate. A commercially distributed IAM.PC.MG 150 × 4.6 mm I.D. column was purchased from Regis (Morton Grove, IL, USA). The column was characterized by particle diameter 12 μm and pore diameter 300 Å.

The chromatographic system consisted of a Model L-6200A pump, a Model L-4250 UV–VIS detector and a Model D-2500 chromato-integrator (all from Merck–Hitachi, Vienna, Austria). The experiments were carried out at ambient temperature using a flow-rate of 1 ml/min.

Test series of steroids and phenols were chromatographed with acetonitrile–0.1 *M* sodium phosphate buffer pH 6.0 (5:95, v/v) eluent. Detection wavelength varied for individual solutes. The compounds studied were injected into the column after dissolution in methanol. In case of the test series of highly ionizable solutes the eluent was pure buffer without organic modifier.

Capacity factors, k'_{IAM}, were calculated assuming as the dead volume of the column a solvent disturbance signal given by methanol.

2.5. Statistical procedure

Quantitative relationships between human skin permeability coefficients and hydrophobicity parameters of the compounds analyzed were studied by a multiple regression analysis method. A standard, commercially available statistical package was employed using a personal computer. Requirements for meaningful regression analysis [15] were observed.

3. Results and discussion

The first set of solutes studied comprised a series of steroid hormones for which the reference hydrophobicity data: *n*-octanol–water partition coefficient (log P_{oct}) and human skin–water partition coefficient (log P_m) were found in the literature [8,13]. Respective data are collected in Table 1 along with the chromatographic hydrophobicity parameter obtained on the IAM column, log k'_{IAM}, and the human skin permeation data, log K_p.

Table 1
Human skin permeability coefficients (log K_p, log P_m) and hydrophobicity parameters (log k'_{IAM}, log P_{oct}) of a group of steroid hormones

No.	Compound	Log K_p [a]	Log P_m [a]	Log k'_{IAM}	Log P_{oct} [b]
1	Aldosterone	−9.08	0.83	0.649	1.08
2	Cortisone	−8.56	0.93	0.772	1.42
3	Deoxycorticosterone	−6.90	1.57	1.730	2.88
4	Estradiol	7.08	1.66	2.179	2.69
5	Estriol	7.95	1.36	1.368	2.47
6	Estrone	−6.00	1.66	2.001	2.76
7	Hydrocortisone	−9.08	0.85	0.843	1.53
8	Pregnenolone	6.38	1.70	2.124	3.13
9	Progesterone	−6.38	2.01	2.199	3.70
10	Testosterone	−6.95	1.36	1.693	3.31

[a] K_p (cm s^{-1}) and P_m according to Scheuplein et al. [8].
[b] According to El Tayar et al. [13].

The relationship between log K_p and log k'_{IAM} for the steroids is given by the equation:

$$\log K_p = -10.19(\pm 0.37) + 1.77(\pm 0.22) \log k'_{IAM}$$
$$n = 10,\ R = 0.942,\ p < 5 \cdot 10^{-5} \quad (1)$$

where n is the number of data points used to derive regression equation, R is the correlation coefficient, p is the significance level and numbers in parentheses denote the standard deviations of individual regression coefficients. Good quality of correlation provided by Eq. 1 is illustrated in Fig. 1a.

The corresponding equation which describes log K_p in terms of log P_{oct} has the form:

$$\log K_p = -10.39(\pm 0.53) + 1.18(\pm 0.20) \log P_{oct}$$
$$n = 10,\ R = 0.900,\ p < 4 \cdot 10^{-4} \quad (2)$$

Correlation between the observed log K_p data and the data calculated by Eq. 2 is illustrated in Fig. 1b. Good statistical quality of both Eqs. 1 and 2 can be explained by the fact that the hydrophobicity parameters, log k'_{IAM} and log P_{oct}, are evidently intercorrelated ($R = 0.911$). However, as evident from Fig. 1 the log k'_{IAM} is a better predictor of skin permeation than log P_{oct}.

For the group of hormones analyzed a highly significant correlation between the skin–water partition coefficient (log P_m) and hydrophobicity parameter determined on the IAM column, log k'_{IAM}, has also been observed:

$$\log P_m = 0.40(\pm 0.10) + 0.64(\pm 0.06) \log k'_{IAM}$$
$$n = 10,\ R = 0.966,\ p < 10^{-5} \quad (3)$$

The corresponding equation describing log P_m in terms of log P_{oct} has the form:

$$\log P_m = 0.33(\pm 0.17) + 0.42(\pm 0.06) \log P_{oct}$$
$$n = 10,\ R = 0.917,\ p < 2 \cdot 10^{-4} \quad (4)$$

Previously Raykar et al. [16] reported correlation between log P_{oct} and log P_m. The high statistical quality of Eqs. 3 and 4 proves that both log k'_{IAM} and log P_{oct} can be used to evaluate the partitioning of steroids between the water and the stratum corneum. Again, the log k'_{IAM} seems to be more reliable for this purpose than log P_{oct}.

The permeability data of 14 phenolic compounds across human epidermis and the reference hydrophobicity data taken from El Tayar et al. [13] are given in Table 2 along with the chromatographic hydrophobicity parameters obtained on the IAM column, log k'_{IAM}.

Eq. 5 describes the parabolic relationship between an epidermics permeability coefficient, log K_p, and the log P_{oct} proposed by the original authors:

$$\log K_p = -8.71(\pm 0.48) - 0.37(\pm 0.10) \cdot (\log P_{oct})^2 + 2.38(\pm 0.46) \log P_{oct}$$
$$n = 14,\ R = 0.943,\ s = 0.22 \quad (5)$$

A lower correlation was observed between log K_p and a chromatographically determined hydrophobicity parameter, log k'_{IAM}:

$$\log K_p = -6.16(\pm 0.20) - 0.46(\pm 0.37) \cdot (\log k'_{IAM})^2 + 1.54(\pm 0.47) \log k'_{IAM}$$
$$n = 14,\ R = 0.797,\ s = 0.40 \quad (6)$$

The linear relationship between log K_p and the hydrophobicity measures log P_{oct} and log k'_{IAM}, have the forms, respectively:

$$\log K_p = -7.20(\pm 0.29) + 0.81(\pm 0.13) \log P_{oct}$$
$$n = 14,\ R = 0.876,\ p < 4 \cdot 10^{-5} \quad (7)$$

$$\log K_p = -6.09(\pm 0.20) + 1.05(\pm 0.26) \log k'_{IAM}$$
$$n = 14,\ R = 0.765,\ p < 10^{-3} \quad (8)$$

In case of this data set log P_{oct} appears to be a better bioactivity predictor than log k'_{IAM}. It has to be noted, however, that a difference between the two hydrophobicity measures is mostly due to the single solute, namely resorcinol. Its log K_p deviates evidently from the remaining solutes. Log P_{oct} appears to better account for this deviation but it may be fortuitous.

The available skin permeation and reference hydrophobicity data for a series of agents known to permeate skin in an ionized forms are col-

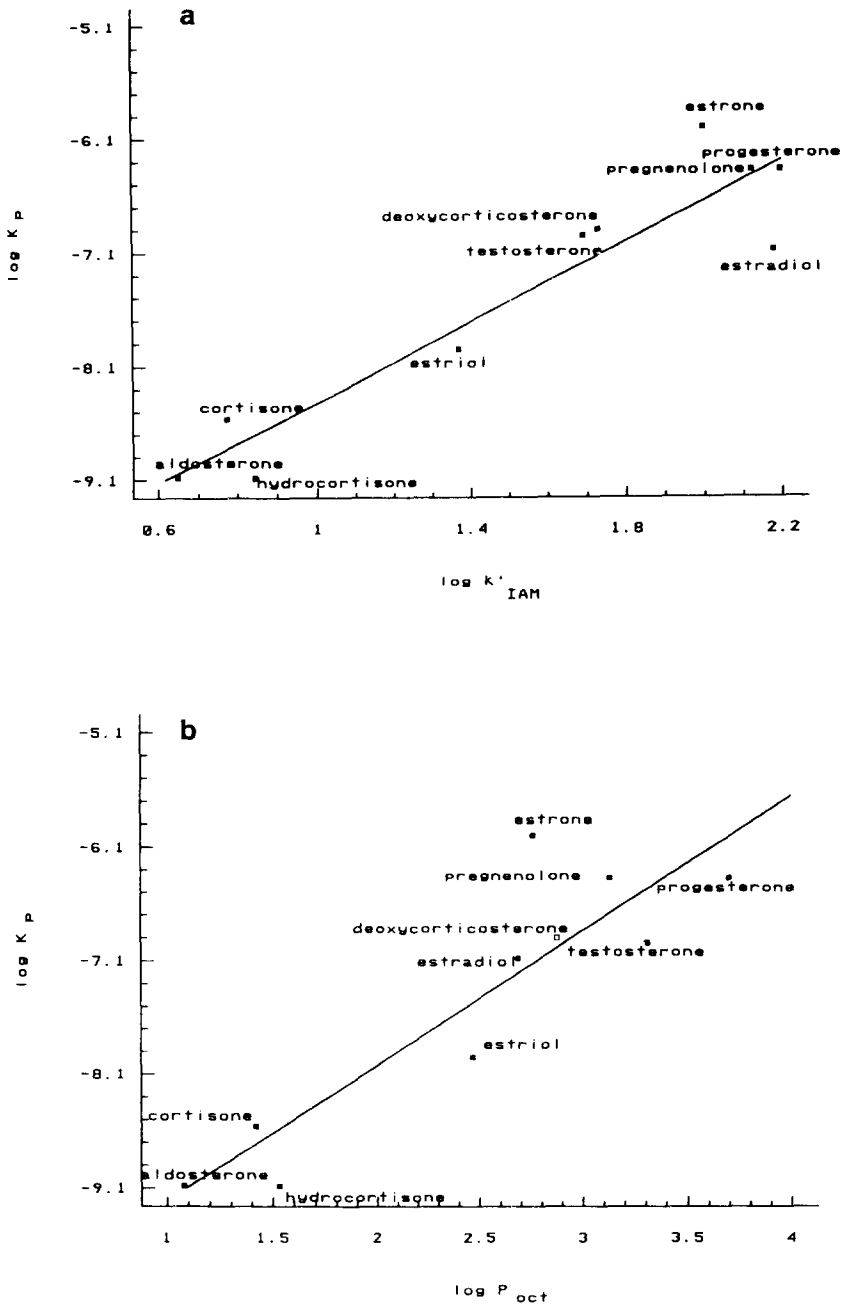

Fig. 1. Correlation between the human skin permeation data of steroid hormones observed by Scheuplein et al. [8], log K_p, and (a) logarithm of chromatographic capacity factor determined on an immobilized artificial membrane column, log k'_{IAM} or (b) logarithm of *n*-octanol–water partition coefficient according to Craig [2], log P_{oct}.

Table 2
Human skin permeability data (log K_p) and hydrophobicity parameters (log k'_{IAM}, log P_{oct}) of phenolic compounds

No.	Compound	Log K_p [a]	Log k'_{IAM}	Log P_{oct} [b]
1	*p*-Bromophenol	−5.00	0.995	2.59
2	Chlorocresol	−4.82	1.183	3.10
3	*p*-Chlorophenol	−5.00	0.728	2.39
4	*o*-Cresol	−5.36	0.363	1.95
5	*m*-Cresol	−5.37	0.363	1.96
6	*p*-Cresol	−5.31	0.418	1.94
7	*p*-Ethylphenol	−5.01	0.761	2.37
8	Methylhydroxybenzoate	−5.60	0.520	1.96
9	β-Naphthol	−5.11	1.254	2.84
10	*m*-Nitrophenol	−5.81	0.598	2.00
11	*p*-Nitrophenol	−5.81	0.595	1.91
12	Phenol	−5.64	0	1.46
13	Resorcinol	−7.18	−0.141	0.78
14	Thymol	−4.83	1.342	3.30

[a] K_p in cm s^{-1} according to Roberts et al. [9].
[b] According to El Tayar et al. [13].

lected in Table 3. Correlation between log k'_{IAM} and log K_p is significant ($R = 0.903$) and that between log P and log K_p is meaningless ($R = 0.136$). Unfortunately, the number of the available data points is highly limited and the correlation observed may be fortuitous.

It is hypothesized that for ionized penetrants another mechanism of skin absorption operates. A porous pathway is supposed and aqueous solubility, not the hydrophobic–hydrophilic partition equilibrium, to determine the rate of skin permeation by ions [11,17].

Hydrophobicity is a complex property depending both on the molecular structure of the solute and on the environment in which it is actually placed. There is no a single, universal hydrophobicity measuring system which could be recommended in every case of structure–bioactivity relationships. Perhaps particular properties of the IAM phases allow for some modeling of skin permeation. Contrary to the standard hydrophobic phases, the IAMs possess polar headgroups being the first site of contact with interacting solutes similarly to natural biomembranes. That first interaction would be of a polar (hydrophilic) nature. If it would be decisive for skin permeation of polar, water-soluble agents then the observed correlation between log k'_{IAM} and log K_p is not by chance but real. It is highly probable, however, that the IAM phase is unable to

Table 3
Human skin permeability data (log K_p) and hydrophobicity parameters (log k'_{IAM}, log P_{oct}) of ionized agents

No.	Compound	Log K_p [a]	Log k'_{IAM}	Log P_{oct} [b]
1	Baclofen	−6.77	−0.725	−0.96
2	Phenylalanine	−8.08	−0.646	−1.35
3	Sodium salicylate	−7.82	−0.575	2.26[c]
4	Tyrosine	−8.14	−0.545	2.26
5	Tryptophan	−8.39	−0.412	−1.04

[a] K_p in cm s^{-1} according to Ruland and Kreuter [10], Mazzenga et al. [11] and Kurihara-Bergstrom et al. [12].
[b] According to Craig [2] and Hansch and Leo [14].
[c] Value reported for salicylic acid.

account for the hydrophobicity differences among readily ionized compounds. Such compounds elute too close to the non-retained markers to make their relative retention data reliable. The correlations reported here will further be tested with the additional skin permeability data currently prepared by us.

In conclusion we would like to emphasize that the new IAM columns allow for a fast and convenient characterization of hydrophobicity of xenobiotics. Our results supply the evidence in support of the hypothesis that HPLC hydrophobicity measures determined on IAM columns may provide different (and more significant in individual cases) input to the description of individual hydrophobicity-affected bioactivity data than the standard hydrophobicity parameters [7]. This could be rationalized in terms of a closer similarity of the IAM structure to the biophase-forming species than in the case of an n-octanol–water system.

Certainly the hypothesis on the general advantage of the HPLC scale determined on IAM columns for molecular pharmacology and pharmacokinetics requires further testing. However, there is an unquestionable advantage of the approach recommended here: it is much easier to obtain precise and reproducible hydrophobicity measures by the means of an IAM column than by the standard shake-flask or by the octanol-like HPLC approaches.

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