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Chromatographic indexes on immobilized artificial membranes for the prediction of transdermal transport of drugs☆

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Abstract

A set of 12 drugs, consisting of structurally unrelated neutral, basic, acidic and amphoteric compounds, was examined by high performance liquid chromatrography (HPLC) on a model of fluid membrane bilayers, the immobilized artificial membrane (IAM) column. The logarithms of chromatographic capacity factors extrapolated to 100% aqueous phase at pH 5.5 ($\log k_w$) were measured and compared to the *n*-octanol/water partition coefficients ($\log P$). The scale derived from the IAM system was different from the lipophilicity scale expressed by the $\log P$, due to the peculiar capability of phospholipids to well accommodate the ionized form of some molecules and show additive or repulsive extra-interactions when particular structural motifs on the molecule are present. The relationship between $\log P$ and $\log k_w$ previously obtained for compounds interacting on IAM phase by a uniquely lipophilicity-based mechanism, allowed us to calculate, from $\log P$, the values of $\log k_w$ expected for the drugs considered. These values were subtracted from the $\log k_w$ experimentally determined and the differences were assumed to quantify the amount of extra-interactions (hydrogen bond and electrostatic interactions) with phospholipids ($\Delta \log k_w$). The coefficients of permeability through the human skin (K_p) for the compounds considered did not correlate with either $\log k_w$ or $\log P$ values. However, the K_p values correlated well with the $\Delta \log k_w$ values indicating that the higher the ability of a molecule to cross the skin barrier, the lower its component of interaction with phospholipids not accounted for by lipophilicity-based interactions.

Keywords: Immobilized artificial membranes; Skin permeation; Lipophilicity

1. Introduction

The introduction of transdermal patch systems capable of delivering therapeutic systemic levels of drugs, can be regarded as the best demonstration that the skin is an alternative route for drug administration. The transdermal penetration of chemicals involves their partitioning into, and transport through, the cutaneous layers, namely the stratum corneum (SC), the stratum basale and the upper dermis; the transport rate in the blood, on the other hand, is rapid and does not limit the process [1]. The SC is thought to be the principal barrier to skin penetration; it is composed of dead cells surrounded by an intercellular matrix of lipids and aqueous layers. The layered structure of the intercellular space is due to the gathering of polar head groups of phospholipids (mainly sphingolipids), with the non-polar chains pointing in opposite directions [2] (Fig. 1). The uptake of penetrants into the SC and the passive diffusion therein are thought to determine the penetration rate of a compound. It has been proposed that chemicals traverse the intact SC by two micropathways: the transcellular route, in which the penetrant diffuses directly through cells, and the intercellular route, in which the penetrant diffuses tortuously in intercellular lipidic phase [3–5]. This last way, involving the crossing of lipophilic and hydrophilic layers is suggested to be predominant. Therefore, this lamellar organization needs to be mimicked by in vitro models able to account for both polar and non-polar interactions.

Solute permeation through a barrier is expressed in terms of permeability coefficient (K_p), i.e. the linear velocity of drug movement through the barrier (cm/s)

☆ Dedicated to Professor Antonio Maccioni.

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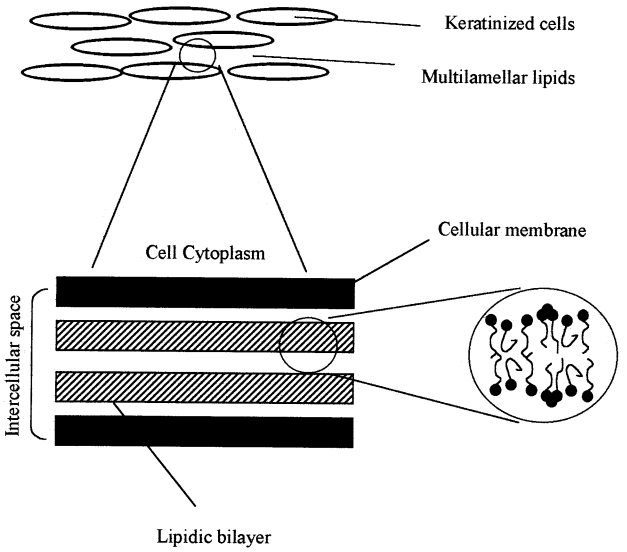

Fig. 1. Diagram of skin penetration of chemicals.

$$K_p = \frac{D_m \cdot K_m}{L} \quad (1)$$

where D_m is the membrane diffusion coefficient, K_m the partition coefficient of the solute in the membrane, and L the thickness of the membrane [6]. Both D_m and K_m values depend on the physico–chemical properties of both solute and membrane. D_m is significantly related to molecular size or molecular weight of drugs. Therefore, the permeation process for solutes spanning over a narrow range of molecular weights is modulated by the different K_m values.

Many membrane systems have been developed to study the transport and measure the permeability of a drug across the skin. In a first attempt, the skin was substituted by synthetic membranes [7] that merely acted as passive diffusional barriers and neglected the role played by drug partitioning into intercellular phospholipids during transport. However, these models resulted inadequate to mimic transdermal passage and, to overcome this limitation, many efforts were devoted to find a proper polymeric substrate containing hydrophobic groups that allowed drug partitioning. Moreover, a variety of skins coming from different animals have been employed [8]. Although these models are too complex to realize and do not take into account all the structural features needed to mimic the physico–chemical properties of skin, they were able to demonstrate the critical role played by the process of drug partitioning into intercellular phospholipid matrix during percutaneous penetration of drugs. The difficulty in taking direct measurements of drug permeability (K_p) by methods based on model membranes has stimulated researchers to attempt its determination by measures of D_m and K_m.

The reference method widely used to evaluate K_m values of a drug is the measure of its partition coefficient in the n-octanol/water system ($\log P$) [9]. In contrast to a bulk phase solvent such as n-octanol, which has invariant properties throughout, the structure of layered phospholipids has very different physico–chemical properties which affect its interactions with drugs, mainly in the case of ionizable compounds. Alternative lipophilic parameters include data from theoretical calculation, CLOGP, and chromatographic capacity factors on hydrocarbon stationary phase by high performance liquid chromatography (RP-HPLC) [10–13]. Although the CLOGP method is widely recognized as the 'industry standard', it has important limitations because the effects of intramolecular interactions (e.g. hydrogen bonds) are often improperly calculated. On the other hand, the HPLC method offers a number of advantages: sample purification is unnecessary, the partition coefficients of a mixture can be measured simultaneously, and only a minimum amount of compound is necessary for the measurements. Unfortunately, due to its high hydrophobicity, the partitioning phase is a poor simulation of membrane structures and cannot model the polar interactions of drugs with phospholipids. This limitation can be overcome by employing new chromatographic stationary phases, the so-called immobilized artificial membranes (IAM), which are composed of monolayers of phosphatidyl–choline covalently bound to a propylamine–silica support made inert by end-capping with methylglycolate [14]. Our studies on some sets of both ionizable or non-ionizable compounds have shown that IAM measures are different from the n-octanol/water partition data, $\log P$, as a consequence of the different nature of the two phases [15–18]. In other words, it seems reasonable that phospholipids, which are of amphipathic nature, can better accommodate hydrophilic compounds than n-octanol, in contrast to a substantially similar interaction capability of the two phases for non-ionizable, highly lipophilic compounds. Since IAM parameters often correlate with biological activity data better than $\log P$, they are also thought to mimic the partition into biomembranes better than $\log P$, the latter only accounting for lipophilicity-driven interactions [19,20]. Therefore, when discrepancies between the scales of the two parameters occur, they could more probably represent a failure of the $\log P$ system to produce effective parameters for the description of the partitioning process of analytes into biomembranes, rather than a drawback of the IAM system.

Moreover, it has been supposed that the tortuous path offered by the HPLC column could effectively model the transport through phospholipid monolayers of SC. Alvarez et al. have demonstrated the effectiveness of IAM parameters to predict the permeability of drugs through human skin [21] for sets of structurally related compounds.

The aim of this work was to evaluate the potential ability of IAM parameters to describe a complex phenomenon such as permeation of drugs through human skin. We determined the IAM interaction data for a set of twelve basic, acidic, amphoteric and neutral compounds. The IAM values were compared to the respective log P measures to ascertain whether the two scales were distinctive. Moreover, the component of interaction on IAM not accounted for by log P was expressed as $\Delta \log k_{w.}$ Finally, the IAM parameters were related to the permeability coefficients to evaluate the role played by the interaction with phospholipids in determining the passage through the skin of the drugs considered.

2. Experimental

2.1. Materials

All samples were obtained from commercial sources. All chemicals were of HPLC grade and used without further purification.

2.2. Chromatographic system

A liquid chromatograph model 600E (Waters–Millipore, Milford, MA) equipped with a model 7125 rheodyne injection valve (fitted with a 20 μl loop) and a model 486 UV detector (Waters) set at wavelength of maximum absorbance for each compound was used. For the detection of isosorbide dinitrate a differential refractometer R401(Waters) was used. The stainless steel column was IAM.PC.MG (4.6 × 150 mm; Regis Chemical Company, Morton Grove, IL). The chromatograms were recorded by a model 746 data module (Millipore).

2.3. Chromatographic conditions

The eluents were mixtures of acetonitrile and 0.10 M phosphate buffer saline (PBS) at pH 5.5, in percentages ranging from 5 to 30%; the flow rate was 1.0 ml/min. The aqueous portion of the eluents was filtered by membrane filters (type HA, Millipore). The eluent mixtures were obtained directly from the chromatographic apparatus by mixing, at low pressure, the organic modifier and the aqueous phase previously degassed by bubbling helium. The chromatography was carried out at room temperature. Samples were dissolved in methanol or water (ca. 10^{-3} M) and a 20 μl sample was injected. Chromatographic retention data are expressed by the logarithm of the capacity factor, log k', defined as $\log k' = \log[(t_r - t_o)/t_o]$ where t_r and t_o are the retention times of the drug and a non-retained compound (methanol), respectively.

All values of log k' are the average of at least three measurements; the 95% confidence interval associated with each value never exceeded 0.04.

2.4. Statistical analysis

A commercially available statistical package for personal computer was used for linear regression analysis. Requirements of significant regression analysis were observed.

3. Results and discussion

Fig. 2 illustrates the structures of the drugs taken into consideration. The set comprises acidic (benzoic acid, salicylic acid, indomethacin, ketoprofen, and naproxen), basic (caffeine, minoxidil), amphiprotic (furosemide, theophylline) and non-ionizable (griseofulvin, hydrocortisone, isosorbide dinitrate) compounds.

Capacity factors on the IAM column (k') were determined with eluents at pH 5.5 in order to perform the measures at experimental conditions as close as possible to the physiological pH of human skin. Furosemide, griseofulvin, indomethacin, ketoprofen, minoxidil and naproxen did not elute within a reasonable time with a completely aqueous mobile phase and the addition of various acetonitrile fractions (φ) to the eluent was needed. A linear relationship between log k' and φ was found for all drugs over the range of the eluent composition examined. Table 1 reports the logarithms of the capacity factors extrapolated to (or measured at) 100% aqueous phase (log k_w). As already observed for other classes of drugs [15,16], differences in elution order occurred at different percentages of the organic modifier. Hence, the normalization of the experimental values to 100% aqueous phase was needed not only to achieve data referred to the partition between phospholipids and water but also to avoid fictitious interaction scales.

To elucidate the mechanism driving the interaction between the drugs considered and the phospholipids, we related the log k_w values to the respective n-octanol/water partition coefficients (log P) (Table 1). The relation equation obtained for the whole set of twelve compounds was quite poor ($r = 0.735$; $s = 0.868$) indicating that the interaction with phospholipids also involves mechanisms other than lipophilicity. To identify the compounds interacting by a uniquely lipophilicity based mechanism, we compared the log k_w measured with the values calculated from log P by the following relation equation, which was previously derived for neutral compounds interacting with IAM phase by lipophilicity driven interactions [16]:

$$\log k_w = 0.816\ (\pm 0.035) \log P - 1.055\ (\pm 0.140) \qquad (2)$$

where $n = 10$, $r = 0.993$, $s = 0.111$.

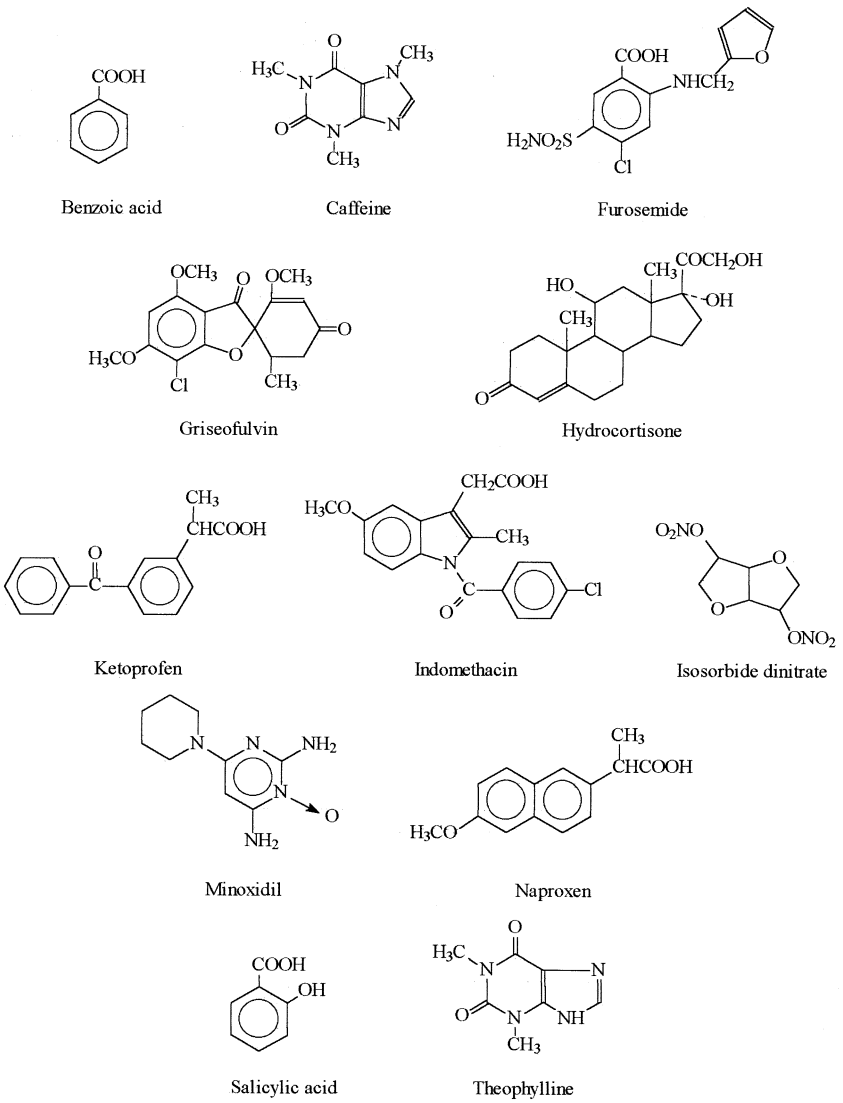

Fig. 2. Chemical structure of the compounds considered.

In this equation and in the following ones, n denotes the number of molecules considered in the derivation of the regression equation, r is the correlation coefficient, and s is the standard error of the estimate. Numbers in parentheses account for the standard error of the regression coefficients.

As can be seen in Table 1, for indomethacin, isosorbide dinitrate, ketoprofen and naproxen, the experimental values of $\log k_w$ were well predicted from $\log P$, indicating that the interaction mechanism of these molecules with phospholipids was mainly lipophilicity driven.

The interaction between isosorbide dinitrate and phospholipids in function of the lipophilicity of the former was as expected, given the fact that it is non-ionizable. Moreover, even the behavior of indomethacin, ketoprofen and naproxen was not surprising since it has already been demonstrated that, at pH values ranging from 5.5 to 7.0, phospholipids can counteract the negative influence of electrically charged functions on the analyte, on the lipophilic interaction. Furthermore, in contrast with the HPLC parameters obtained on hydrocarbon stationary phases, IAM measures and $\log P$ values were on a single scale when considering both neutral and ionizable structurally unrelated molecules [16].

The interaction between IAM and the other compounds, indicated as outliers, also included mechanisms not accounted for by the $\log P$.

In contrast with the other acidic compounds, benzoic and salicylic acids interacted with phospholipids to a smaller extent than expected for neutral isolipophilic compounds. This could be due to the presence of a carboxylic function directly linked to the aromatic ring, as this structural feature was already recognized to disturb to a constant extent the lipophilic interaction with phospholipids [16].

Table 1
Lipophilicity parameters, measured and calculated IAM capacity factors

	log P	log k_w	log k_{cal}
Benzoic acid	1.88	−0.222	0.458
Caffeine	0.07	0.021	−1.028
Furosemide	2.29	1.615	0.795
Griseofulvin	1.95	1.975	0.516
Hydrocortisone	1.86	1.503	0.442
Indomethacin	4.23	2.364	2.388
Isosorbide dinitrate	1.12	−0.146	−0.165
Ketoprofen	2.79	1.341	1.206
Minoxidil	1.23	0.432	−0.075
Naproxen	2.82	0.981	1.230
Salicylic acid	2.27	0.394	0.779
Theophylline	−0.25	−0.101	−1.189

For basic and amphiprotic molecules, viz. caffeine, furosemide, minoxidil and theophylline, the interaction with phospholipids was stronger than that predicted from log P. This behavior was in agreement with the unique capability of phospholipids to accommodate the protonated form of compounds supporting an aminic function [15,17,18], confirming that for basic compounds the presence of a positive charge on the molecule not only was counteracted by phospholipids but even enhanced the interaction. Unfortunately, so far no final indication about the structural features allowing this phenomenon can be derived.

Surprisingly, two neutral molecules, griseofulvine and hydrocortisone, also showed log k_w values higher than expected. The reasons for this particular behavior are not clear and could be related either to the planarity of the molecules, which could favor the intercalation within the phospholipidic layer, or to the presence of alcoholic and carbonylic functions acting as donor and/or acceptor of hydrogen bonds [22].

Therefore, we can conclude that for the set of compounds considered the IAM parameters gave an interaction scale with phospholipids which was distinctive from that of log P values.

Previous correlative studies between data of biological activity involving interactions with biomembranes and in vitro descriptors [15–17,23] demonstrated that IAM parameters perform better than log P in mimicking the in vivo interactions with phospholipids.

The passage through the skin is a complex phenomenon that certainly involves the interaction between penetrants and phospholipids, although the importance of this aspect is still unclear. Due to the lack of correlation between log P and log k_w scales for the compounds considered, we attempted to verify whether the IAM parameters could be more effective than log P in predicting this passage. As a matter of fact, the knowledge of the influence of chemical structures on skin permeability can serve two purposes. First, such information is essential for identifying the transport mechanism involved and characterizing the nature of the barrier microenvironment. Second, a quantitative description relating physico–chemical properties to skin permeability would be most valuable from a practical standpoint for the prediction of the permeation rate of new drug candidates or as a guide in the design of molecules with enhanced penetration capability.

In this study we considered the values of transdermal flux measured by classical in vitro diffusion experiments on excised skin [24].

The transdermal fluxes can be predicted by models based on physico–chemical properties of penetrants such as partition coefficient and water solubility. A model relating the partition coefficient values to the total flux (J) is expressed by the following equation [25]:

$$J = \frac{36 C_w}{2.82 + (29.6/P)} \quad (3)$$

where C_w is the water solubility and P is the n-octanol/water partition coefficient of the drug.

No correlation between the experimental values and those predicted by the above equation was found for our compounds. The substitution of P values with k_w parameters, not intercorrelated, was attempted in the supposition that the IAM measures are probably better descriptors of partition in biological barriers. However, no improvement in the relationship was obtained (equations not shown). Therefore, in both cases this simple mathematical model was not effective for the prediction of flux values. It is important to remember that this model does not include any parameter accounting for molecular volumes that could be considered roughly collinear to the P values only within structurally homogeneous classes of compounds. In fact, many studies have demonstrated that for series of structurally homogeneous compounds the permeation coefficients K_p, obtained from total flux values corrected on water solubility of the drug, are well related to log P and still better predicted from interaction parameters on IAM [21,26].

Actually, we did not find any correlation between K_p and k_w values for the compounds considered and no improvement was obtained by performing the correction of k_w values on molecular weights, this last procedure being sometimes necessary when mimicking a passage through a barrier to take into account in some way the influence of molecular volume. Therefore we can infer that the transport process across the skin is affected by various physico–chemical parameters whose contribution is different for series of heterogeneous drugs.

Table 2
Water solubilities, total transdermal fluxes, permeability coefficients and differences between experimental and calculated IAM values

	C_w [a]	J [b]	log K_p [c]	$\Delta \log k_w$
Benzoic acid	0.5	720	−0.40	−0.680
Caffeine	22	1.5	−4.72	1.049
Furosemide	0.54	0.21	−3.97	0.820
Griseofulvin	0.014	0.24	−2.32	1.459
Hydrocortisone	0.28	0.42	−3.38	1.061
Indomethacin	0.016	0.25	−2.36	−0.024
Isosorbide dinitrate	1.09	4.8	−2.91	0.019
Ketoprofen	2.97	12	−2.95	0.135
Minoxidil	1.9	0.81	−3.93	0.507
Naproxen	0.016	4.8	−1.08	−0.249
Salicylic acid	0.002	1.9	−0.58	−0.385
Theophylline	7.5	1.08	−4.40	1.189

[a] C_w (water solubility) in units of mg/ml (from Ref. [24]).
[b] J (total transdermal flux) in units of mg/cm^2 per h (from Ref. [24]).
[c] K_p in units of cm/s.

El Tayar et al. [5] found good relationships between $\Delta \log P_{oct-hep}$ and log K_p for some series of alcohols and steroid hormones. $\Delta \log P_{oct-hep}$ values express the difference between *n*-octanol/water partition coefficients and heptane/water partition coefficients and are believed to assess hydrogen-bond donor acidity of solutes [27,28]. The use of this parameter, in contrast to log P, allowed us to include in a unique relationship different series of compounds. The relationship between $\Delta \log P_{oct-hep}$ and log K_p had a negative slope, indicating that compounds with higher $\Delta \log P_{oct-hep}$ values permeated poorly. Analogously, we calculated the differences between the log k_w values measured and those predicted from log P by Eq. (2) ($\Delta \log k_w$) (Table 2). This value represents the interaction component of a solute with phospholipids not accounted for by the lipophilicity measures in the *n*-octanol/water system. In the case of ionized molecules, $\Delta \log k_w$ express mainly attractive or repulsive interactions of electrostatic nature between the solute and the charged head groups of phospholipids [15–17], although some contribution of hydrogen-bond donor and/or acceptor capability of the molecules cannot be excluded. In fact, it has recently been reported [22] that for non-ionizable molecules it is mainly their hydrogen-bond capability that determines the discrepancies observed between data obtained from IAM and from *n*-octanol systems.

The plot of log K_p versus $\Delta \log k_w$ values is shown in Fig. 3. A reasonable relationship was found for ten compounds:

$$\log K_p = -2.419(\pm 0.276)\,\Delta \log k_w - 2.206(\pm 0.174) \quad (4)$$

where $n = 10$, $r = 0.952$, $s = 0.517$.

Griseofulvin and hydrocortisone were strong outliers and were not included in the equation. It is important to remember that they are the only non-ionized compounds showing additive extra-interactions with phospholipids. Therefore, their peculiar behavior cannot be referred to mechanisms related to electrostatic interactions but probably arises from both hydrogen bonding capability and the structured nature of phospholipidic stationary phase.

Eq. (4), including structurally unrelated compounds, indicates that the component of electrostatic interaction with phospholipids, which is not encoded in the log P measure, strongly affects the permeability of the drugs.

In contrast with the results of El Tayar et al. [5], the introduction of log P parameter into Eq. (4) did not improve the correlation. Worthy of note is that $\Delta \log k_w$, and log P were not intercorrelated ($r = 0.612$):

$$\log K_p = -2.136(\pm 0.392)\,\Delta \log k_w + 0.037(\pm 0.183)\log P - 2.373(\pm 0.443) \quad (5)$$

where $n = 10$, $r = 0.936$, $s = 0.586$.

Similar results were observed by the introduction of log k_w in Eq. (4). Again, log k_w did not correlate with either $\Delta \log k_w$ or log P scales ($r = 0.054$ and 0.823, respectively):

$$\log K_p = -2.182(\pm 0.311)\,\Delta \log k_w + 0.046(\pm 0.183)\log k_w - 2.323(\pm 0.252) \quad (6)$$

where $n = 10$, $r = 0.936$, $s = 0.586$.

This indicates that, considering a set of structurally unrelated compounds, neither log P nor log k_w values are a measure of positive driving forces affecting skin permeation. This could depend on the fact that the compounds strongly differ in their diffusion coefficient values (D_m). An alternative explanation arises from the

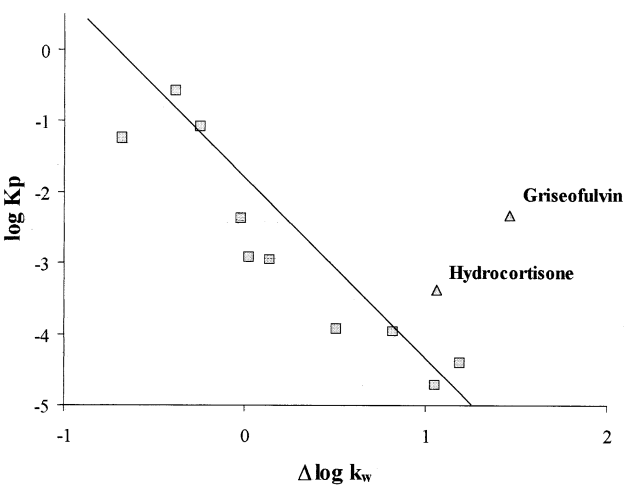

Fig. 3. Plot of log K_p (permeability coefficient, cm/h) vs. $\Delta \log k_w$ for all compounds.

observation that the good relation equation between $\Delta \log k_w$ and $\log K_p$ is relative to a set including ionized molecules, being only isosorbide dinitrate a neutral compound having $\Delta \log k_w$ equal to zero. In this case the influence of electrostatic interactions, whether negative or positive, appears to play a major role in the permeation process which depends, to a negligible extent only, on other physico–chemical properties such as partitioning into phospholipids. Therefore, the penetrants somehow repulsed from phospholipids showed high penetration coefficients, whereas molecules whose affinity with phospholipids was very high, encountered a difficulty in crossing the skin.

The results of the present work confirm that IAM chromatography is a rapid and effective technique to obtain physico–chemical parameters useful for the prediction of biological data, mainly when ionized compounds are considered. On the other hand, the results of the present work indicate that IAM and log P values are complementary and not alternative parameters whose combination can provide further insight into the interaction mechanisms between drugs and biological barriers.

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