Original Research

Physicochemical properties and permeation across mouse skin of non-steroidal anti-inflammatory drugs

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Abstract

The skin provides an effective barrier and only limited number of drugs can penetrate in adequate amounts. This study aims to identify the relationship between the physicochemical properties and permeation across mouse skin of non-steroidal anti-inflammatory drugs in view of their feasibility to transdermal delivery. Biphenylacetic acid, diclofenac base, diclofenac sodium, indomethacin and piroxicam are the drugs studied. Number of physicochemical properties studies were performed. Drug permeation studies across hairless mouse skin were carried out using an *in-vitro* finite dosing diffusion cell. The relationship between physicochemical properties of the drugs studied and their percutaneous penetration was studied. Purity for the compounds studied ranged from 99.09% to 100% in which 99.89% purity was obtained for diclofenac base. At 7.4, the % ionized of piroxicam was found to be 95.12%, while for other drugs were in the range of 98.01% and 99.96%. The true partition coefficient values in the *n*-octanol/water system are in the range of 1.85 and 2.85, while in the *n*-octanol/phosphate system ranged from 2.14 to 3.70. Observed solubility in water, phosphate buffer and *n*-octanol ranged from 0.033 to 0.322, 0.202 to 0.329 mg per ml, and 2.19 to 16.10 mg per ml, respectively. A linear relationship was found between water solubility and melting point between steady-state rates of permeation across intact and viable skin, between *n*-octanol solubility and maximum predicted flux and between molecular volume and the ratio of maximum predicted flux. Predicted flux calculated was compared with the experimental data which resulted in a high correlation. Physicochemical criteria which were determined the feasibility of non-steroidal anti-inflammatory drugs studied for transdermal delivery were identified. The relationships obtained in this study provide an essential physical and chemical properties that govern transport of non-steroidal anti-inflammatory drugs across hairless mouse skin.

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Keywords: Non-steroidal anti-inflammatory drugs, mouse skin, partitioning, physicochemical properties, solubility, transdermal delivery

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Introduction

Transdermal delivery of drugs is a major area of interest to pharmaceutical scientists. Due to higher compliance and avoiding of possible side-effects of other routes, the idea of administering a drug transdermally in a delivery device is to release a constant and continuous effective dose to target tissue through controlled-release mechanism. Transdermal route of administration cannot be employed for all types of drugs. It depends upon optimal physicochemical properties of the drug and its biological properties [1]. The evaluation of drug transport across biological membranes is a central area of interest in the pharmaceutical sciences. Physicochemical factors alter the availability and thus the activity of the drug by controlling the transport across biological membranes. The skin acts as a reservoir that deliver the drug, thus, reduces possible toxicity and avoiding systemic side effects [2]. The primary resistance to the drug permeation resides in the

stratum corneum. The stratum corneum of the skin may act as a barrier that limiting passage of drugs to cross the skin, however, if a drug is highly lipophilic and/or metabolized in the viable skin. The physicochemical interactions of the drug with the viable skin may play an important role in transdermal drug delivery [3]. It has been suggested that solubility and diffusivity of the penetrant in the skin are the critical factors which control the rate of drug permeation across the skin [4]. Although diffusivity and solubility of drugs may be generated from the full-thickness skin specimen (single-layer skin model) as a first approximation in the analysis of skin permeation data, two-layer skin model (the stratum corneum and viable skin) is proposed in which the physicochemical properties determined in each layer can be used to analyses the pharmacokinetics of percutaneous absorption [4]. Oral administration of non-steroidal anti-inflammatory drugs (NSAIDs) is known to cause gastrointestinal complications which is an important factor that limits their use orally [5]. However, the use of topical NSAIDs has the advantage of a lower risk of gastrointestinal toxicity and other undesirable side effects [6]. Most NSAIDs are absorbed and exhibit efficacy when applied topically in simple solutions, ointments or creams [7]. The use of transdermal delivery for pharmaceuticals has been limited because only few drugs have proven to be effectively delivered through the skin [8]. In the context of the development of transdermal therapeutic systems (TTS), NSAIDs have received a little attention, therefore, it was an objective to provide a progress work in this area. At the present investigation, some NSAIDs were chosen, according to their properties which are important for transdermal delivery, to study the relationship between the physicochemical properties of these drugs and their permeation across the skin towards transdermal drug delivery.

Materials and methods

Drugs and physicochemical studies: Biphenyl acetic acid (Whelehm, Son & company Ltd., Ireland), diclofenac sodium, indomethacin and piroxicam (Sigma chemical, U.K) were purchased and used in this study. Micro-analysis studies of drugs including diclofenac base which were prepared from diclofenac sodium were carried out by IR and NMR analysis. Purities and melting points of the NSAIDs were determined. Melting points of the studied compounds were determined using digital melting point apparatus (INE-WRS-1B). The techniques used for drug-purity determinations were based on modification of classical Pregl-Dumas method [9]. The ionization constant (pK$_a$) was determined by spectrophotometric method [10] and approximate value was initially estimated. Using this approximation of pK$_a$, seven buffer solutions were prepared with pH values within ± 0.6 units of the estimated values. A set of seven values of pK$_a$ was then obtained from measurements of the absorbance of those solutions which pK$_a$ value was determined. The solubility of each compound was determined in double-distilled water, phosphate buffer solution (pH 7.4) and n-octanol. Partition coefficient (P) was performed in n-octanol/water and n-octanol/phosphate buffer (pH 7.4) systems. From the observed solubility in distilled water (S), the intrinsic solubility (S_o) was derived [10]. The observed partition coefficient (P_{obs}) of each compound was calculated and the true or corrected partition coefficient (P) was obtained accordingly [11].

Skin permeation procedures: Male hairless mice of 5 - 7 weeks-old (Bioresourses Unit, TCD, Ireland) were sacrificed. Either the intact skin of full-thickness or stripped skin was used. Taking care not to damage the skin, the dermal side was cleaned of any adhering subcutaneous tissue with blood vessels. To obtain the stripped skin, the abdominal surface of the mouse was stripped repeatedly with cellophane tape (3M Co., Germany) in which the tape was placed firmly against the surface and peeled away 25 times using fresh piece of cellophane tape each time. Both full-thickness and stripped skin specimens were employed immediately after preparation. Permeation studies were carried out using an *in-vitro* finite dosing diffusion cell. The cell used is a modification of the Keshary-Chien diffusion cell [12]. In the modification, sampling is improved with an addition of the sampling port at the side of the vessel. Preparation of saturated solution of each drug in phosphate buffer (pH 7.4), skin samples and skin permeation procedures were applied according to previously published methods [13]. The drug concentration in the sample solution was analyzed by UV spectroscopy at 231, 275, 276, 261 and 355 nm wavelength for BPAA, diclofenac, diclofenac sodium, indomethacin and piroxicam, respectively (each study was repeated 4 times). The skin permeation profiles were constructed by plotting the cumulative amount (Q) of drug penetrated against time. The lag time (T_{lag}) was then obtained at the intercept on the time axis by extrapolating from the steady-state permeation profile. The steady-state rate of permeation was calculated from the linear slope of the permeation profile. The solubility

and diffusivity of the studied NSAIDs in each skin layer were calculated from the lag time values and the steady-state rates of permeation across the intact and stripped skin. The solubility in the skin based on the two-layer skin model was determined using the approach proposed by Tojo and others [4]. The drug solubility in the stratum corneum (C_2) was calculated and the drug diffusivity across the stratum corenum (D_2) was obtained. The possibility of establishing relationship between the physicochemical properties of drugs studied and their percutaneous penetration was examined. The molecular volume (V_m) of each compound was calculated, where the steady-state flux (J_{ss}) through homogeneous membrane was calculated. The maximum penetration flux rates were calculated using the membrane solubility of the penetrant (S) based on the solution theory method [14], while the ideal solubility (S_{ideal}) in skin lipids was estimated. [15]. The ideal solubility was calculated using σ = 900 gm per liltre, M_1 = 300 Daltons, T = 303 Kelvin (^oK), and ΔS_f = 16 EU (σ, M_1, and ΔS_f values were obtained from literature). To predict skin penetration, the dependence of the diffusion coefficient in stratum corenum (D) was calculated. The diffusion parameter (P_1) and the partition parameter (P_2) are defined by equations proposed by Okamoto et al. [16]. The total amount of penetrant appearing in the receptor solution in time t (Q_t) were calculated [17]. The permeability constant (K_p) and the lag time (T_{lag}) were calculated.

The parabolic relationship between values of log K_p and their corresponding log P was obtained. Using the data predicted, the mean flux (J) was calculated and the amounts permeated in 24 hours (Q_{24}) were calculated. For a candidate to be formulated in TTS design, the predictive parameters were calculated.

Statistical analysis

Statistical regression analysis was performed by using Microsoft Excel 97-2003 Worksheet and the significance level was set at $p < 0.05$.

Results

Drug microanalysis: Diclofenac base that was prepared from diclofenac sodium was a fine, white powder. IR and NMR spectra for both compounds was demonstrated and well established, see (***supplementary***).

Physicochemical properties: The melting point, purity, ionization constant (pK$_a$) and percent ionized are presented in **Table 1**. Determination of (pk$_a$) for the NSAIDs studied reveals that ranges from 3.96 to 6.11 (***supplementary***). The percentage of purity for the compounds studied ranged from 99.09 to 100% in which 99.89% purity was obtained for diclofenac base, the product that was prepared at the present study. At physiological pH 7.4, the percent ionized of piroxicam was calculated to be 95.12 %, while for the other four drugs were in the range of 98.01 - 99.96%. The log P values in the *n*-octanol/water system are in the range of 1.85 - 2.85, while in the *n*-octanol/phosphate buffer system ranged from 2.14 to 3.70. The solubility in distilled water, phosphate buffer solution (pH 7.4) and *n*-octanol produced linear relationship for each drug in the three systems when absorbance values were plotted against drug concentration (***supplementary***).

Table 1: Melting point, purity, pK$_a$ and percent ionized of NSAIDs studied

Drug	Melting point (ºC)	Purity (%)	pK$_a$	% ionized	
				pH 5.6	pH 7.4
BPAA*	168 – 174	100	3.96	97.76	99.96
Diclofenac	179 – 183	99.89	5.12	75.12	99.50
Diclofenac Na	286 – 292	99.09	5.71	43.70	98.01
Indomethacin	162 – 168	100	4.66	89.79	99.80
Piroxicam	199 – 205	99.55	6.11	23.61	95.12

*Biphenylacetic acid

The solubility and partition coefficient of NSAIDs studied are given in **Table 2**. The observed aqueous (water) solubility ranged from 0.033 to 0.322 mg per ml. BPAA has the lowest solubility while diclofenac sodium was found to be the most soluble drug. The melting point (m.p.) values of the drugs studied were correlated with aqueous solubility.

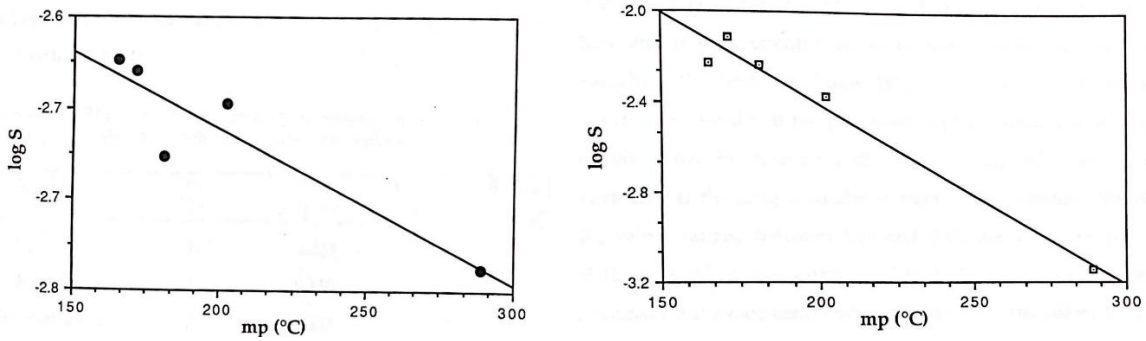
Figure 1A showed linear relationship between water solubility and melting point (*log S = -2.555-0.001 m.p., r = 0.919, p < 0.05*). The solubility studies in phosphate buffer, pH 7.4 were between 0.202 and 0.329 mg per ml in which correlation was found with the m.p.

Figure 1B showed linear relationship between phosphate buffer solubility and m.p. (*log S = - 0.796 - 0.008 m.p., r = 0.987, p < 0.01*). The measured solubility in *n*-octanol ranged from 2.19 to 16.10 mg per ml.

Table 2: Solubility and partition coefficient of NSAIDs studied.

Drug	Solubility (mg ml^{-1})				Partition coefficient (log *P*)	
	Water, *p*H 6.8		Buffer[b]	*n*-octanol	*n*-octanol/water	*n*-octanol/buffer
	S	S_o				
BPAA[c]	0.033	4.763 x 10^{-5}	0.234	16.10	2.84	3.23
Diclofenac	0.110	2.251 x 10^{-3}	0.248	12.06	2.68	2.92
Diclofenac Na	0.322	2.435 x 10^{-1}	0.329	2.19	1.85	3.14
Indomethacin	0.044	3.165 x 10^{-4}	0.302	12.58	2.71	3.70
Piroxicam	0.039	6.613 x 10^{-3}	0.202	8.12	2.61	2.14

[a]S is the observed solubility and S_o is the intrinsic solubility; [b]Fresh buffer, *p*H 7.4; [c]Biphenylacetic acid



(A) **(B)**

Figure 1: The relationship between the observed water solubility (A) and phosphate buffer solubility (B) in mole fraction (log *S*) and melting points of NSAIDs studied.

Drug stability: The stability of the NSAIDs in phosphate buffer solution either from that kept in contact with the dermis of mouse skin or from that of the fresh solution (no contact with skin) are shown in **Table 3**. The observed rate constants were obtained from the plots of concentration versus time. The correlation coefficient for all conditions ranged from 0.965 to 1.000.

Table 3: Rate constants for the degradation of NSAIDs in phosphate buffer solution.

Drug	Rate constant x 10^{-2} (hr^{-1})	
	Solution A[a]	Solution B[b]
BPAA[c]	2.099	2.149
Diclofenac	2.134	2.409
Diclofenac Na	2.553	2.305
Indomethacin	2.913	2.213
Piroxicam	2.616	2.468

[a]Phosphate buffer kept in contact with the dermis of the mouse skin overnight, [b]Fresh phosphate buffer, [c]Biphenylacetic acid

Drug solubility and diffusivity: The solubility and diffusivity of NSAIDs studied in the intact and the viable skin, which were calculated, are listed in **Table 4**. The effect of drug solubility in the stratum corenum and in the viable skin on the steady-state rate of permeation is shown in **Figure 2**. A linear relationship (r = 0.954 for the intact skin and r = 0.927 for the viable skin) was established between steady-state rates of permeation across each skin layer and the drug solubility.

Table 4: Solubility and diffusivity of NSAIDs in the hairless mouse skin

Drug	Solubility (mg ml^{-1})		Diffusivity (cm^2 sec^{-1})	
	SC[a]	VS[b]	Sc. 10^{-12}	VS.10^{-12}
BPAA[c]	23.25	0.66	97.10	5.69
Diclofenac	11.89	0.42	20.16	3.60
Diclofenac Na	8.24	0.37	1.32	5.84
Indomethacin	5.75	1.39	11.42	6.25
Piroxicam	18.24	0.88	28.60	4.45

[a]Stratum corenum, [b]Viable skin, [c]Biphenylacetic Acid

Skin penetration profiles: Permeation profiles were constructed by plotting the cumulative amount (Q) of NSAIDs studied against time across the intact and stripped skin (**Figure 3**). The experimental data on the permeation rates and lag time (T_{lag}) values across the intact and the stripped skin are summarized in **Table 5.**

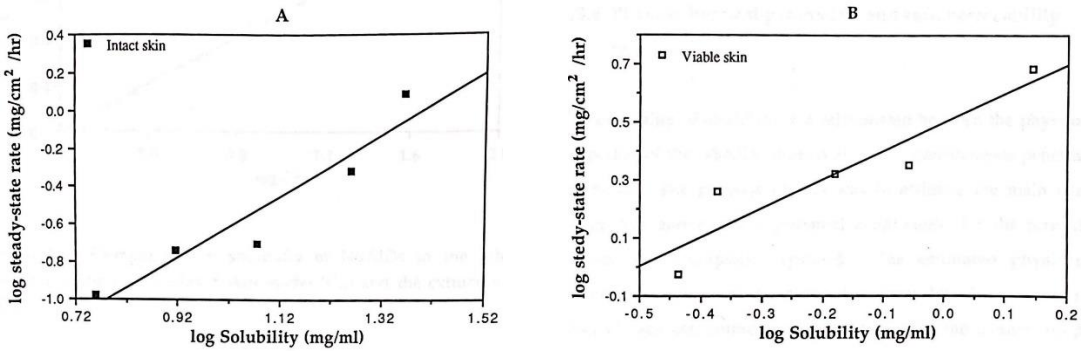


Figure 2: Effect of drug solubility in the stratum corenum and in the viable skin on the steady-state rate of permeation across the intact skin (A) and the viable skin (B).

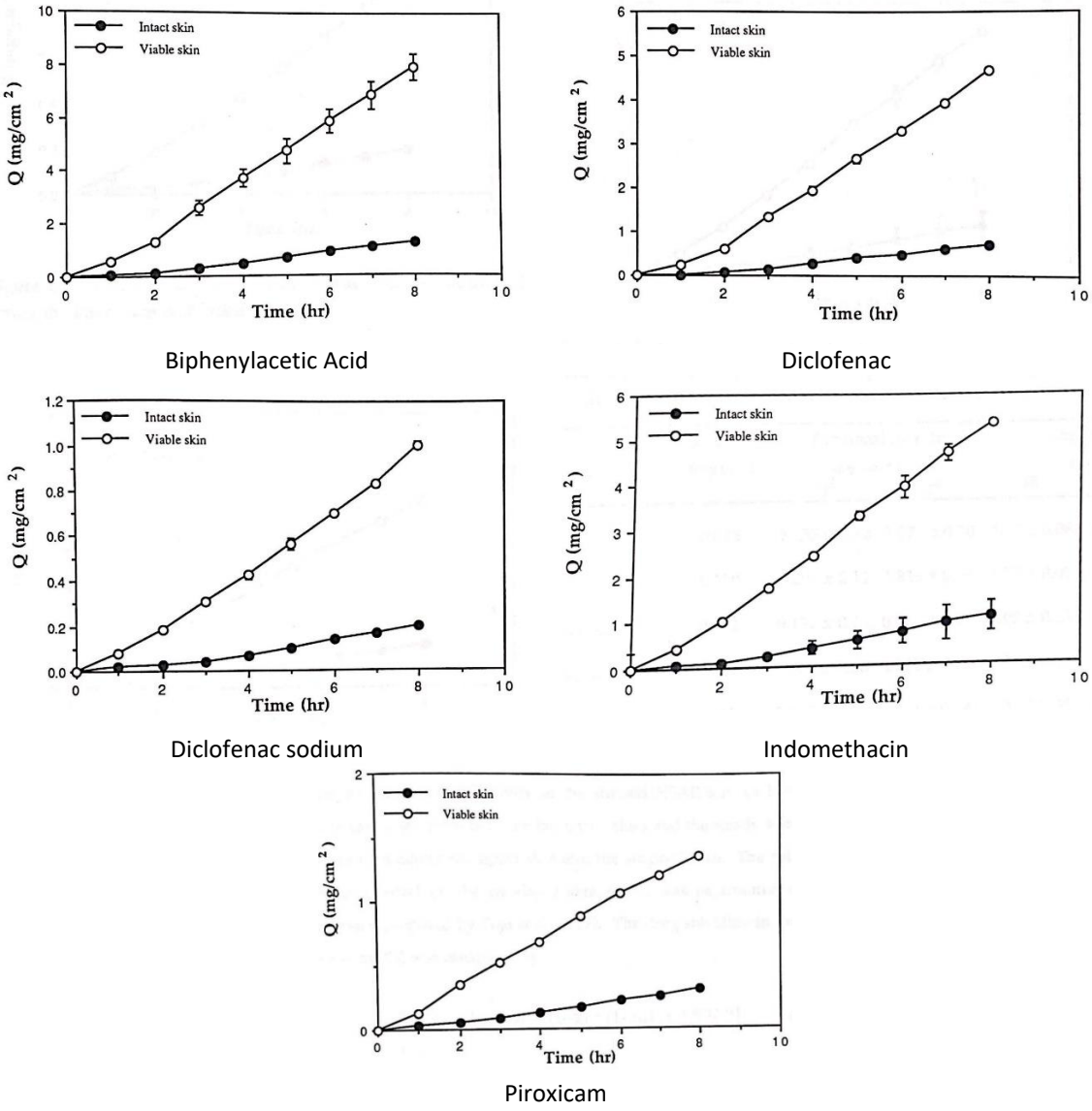


Figure 3: Permeation profile of studied NSAIDs (mean ± SD, *n* = 4) across the intact and viable skin

Table 5: The steady-state of penetration and lag time values of NSAIDs across the hairless mouse skin (mean ± SD, n = 4)

Drug	Solubility[a] (mg ml^{-1})	Permeation rate (mg cm^{-2}hr^{-1})		Lag time (hr)	
		IS[b]	SS[c]	IS[b]	SS[c]
BPAA[d]	0.033	1.233 ± 0.12	2.078 ± 0.10	1.57 ± 0.08	0.63 ± 0.08
Diclofenac	0.110	0.202 ± 0.11	1.819 ± 0.03	1.75 ± 0.01	0.99 ± 0.30
Diclofenac Na	0.322	0.182 ± 0.13	0.946 ± 0.03	2.02 ± 0.25	0.61 ± 0.05
Indomethacin	0.044	0.106 ± 0.18	4.821 ± 0.16	1.57 ± 0.10	0.57 ± 0.17
Piroxicam	0.39	0.487 ± 0.14	2.231 ± 0.14	1.20 ± 0.35	0.08 ± 0.02

[a]Determined in phosphate buffer solution (*p*H 7.4), [b]Intact skin, [c]Stripped skin, [d]Biphenylacetic Acid

Physicochemical properties and skin permeability relationship: The estimated physicochemical parameters and predicted maximum fluxes of NSAIDs studied are summarized in **Table 6**. A linear relationship was demonstrated between *n*-octanol solubility and m.p., between *n*-octanol solubility and maximum predicted flux and the correlation relationship between molecular volume and the ratio of maximum predicted flux and *n*-octanol solubility. The following regression equations were produced for each, respectively, (SE = standard error):

$$\log S = 1.7146 - 0.0035\,(m.p)$$
$$P < 0.05 \qquad r = 0.933 \qquad s = 0.08$$
$$\log J_m = -3.5853 + 3.1184\,(\log S)$$
$$P < 0.05 \qquad r = 0.696 \qquad s = 0.715$$
$$\log \frac{J_m}{S} = 2.096 - \frac{0.013}{2.303}\,(V_m)$$
$$P < 0.05 \qquad r = 0.638 \qquad s = 0.772$$

BPAA with the lowest molecular volume of the series (V_m = 194) resulted in the predicted flux of 3.027 mg cm^{-2} per hour, while diclofenac sodium and indomethacin, where their V_m are the highest among drugs studied (279 and 307, respectively), showed lower predicted values of 0.071 and 0.028 mg cm^{-2} per hour. The maximum predicted flux for piroxicam (V_m = 253) and diclofenac (V_m = 238) are 0.620 and 1.366 mg cm^{-2} per hour, respectively. When the ratio of the maximum predicted flux and the ideal solubility was plotted against the molecular volume, the obtained relationship showed similar trend, but resulted in better fit (r = 1.00). Similarly, the ratio was plotted against the molecular weight of the five NSAIDs studied. The resulting regression equations for both relationships are:

$$\log \frac{J_m}{S} = 1.081 - \frac{0.008}{2.303}\,(V_m)$$
$$P = 0.001 \qquad r = 1.000 \qquad s = 0.002$$
$$\log \frac{J_m}{S} = 0.766 - \frac{0.006}{2.303}\,(MW)$$
$$P < 0.05 \qquad r = 0.929 \qquad s = 0.151$$

Furthermore, the predicted flux calculated was then compared with the experimental data (**Table 5**) which resulted from *in vitro* penetration studies in the full-thickness hairless mouse skin. From the relationship shown in **Figure 4**, a high positive correlation (r = 0.891) was obtained between experimental and predicted flux. The resulting regression equation is

$$J = 0.1013 - 0.3331\,(J_m)$$
$$P < 0.05 \qquad r = 0.891 \qquad s = 0.244$$

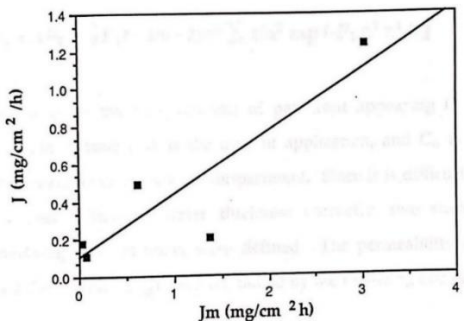


Figure 4: Correlation between the maximum predicted flux (J_m) and the experimental obtained data (J).

Table 6: Estimated physicochemical properties and predicted maximum fluxes of NSAIDs

Drug	MW (Dalton)	m.p (°C)	V_m (Å^3)	Log *P*	$S_{octanol}$ (g L^{-1})	S_{ideal}	J_m (mg cm^{-2} h^{-1})
BPAA[a]	212	168 – 174	194	2.84	16.10	10.17	3.027
Diclofenac	296	179 – 183	238	2.68	12.06	10.58	1.366
Diclofenac Na	318	286 – 292	279	1.85	2.19	0.48	0.028
Indomethacin	358	162 – 168	307	2.71	12.58	2.05	0.071
Piroxicam	331	199 – 205	253	2.61	8.12	6.39	0.620

[a]*Biphenylacetic Acid*

Effect of partition coefficient on drug diffusion: The estimated mean *Kp* values are summarized in **Table 7**. It was in the range of 2.06 x 10^{-3} to 5.70 x 10^{-3} cm^2 per hour. The parabolic function was found to fit the log K_p values using the parameter log *P*. The parabolic relationship is shown in **Figure 5** and the following equation was obtained:

$$Log\ K_p = -5.242 + 1.757\ (log\ P) + 0.26\ (log\ P)^2$$

$P < 0.05$ $r = 0.973$ $s = 0.083$

Table 7: The estimated permeability constants for the NSAIDs studied using equation (12).

Drug	Kp* ($cm^2\ h^{-1}$ x 10^{-3})
Biphenylacetic Acid	5.39 ± 0.7
Diclofenac	3.73 ± 0.1
Diclofenac sodium	2.06 ± 0.2
Indomethacin	5.70 ± 0.3
Piroxicam	2.31 ± 0.1

*Data expressed as mean ± SD, n =4

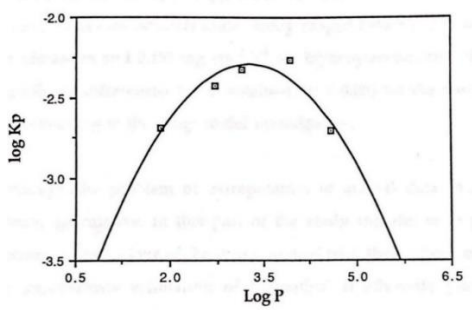


Figure 5: The parabolic relationship between log K_p and corresponding log *P* values for the NSAIDs studied

Prediction of parameters related to TTS formulations: The relevant parameters estimated for the formulation of TTS was tabulated in **Table 8**. Biphenylacetic acid and indomethacin displayed the highest predicted plasma levels at a steady-state concentration (C_{ss}). The predicted permeated amounts during 24 hours (PQ_{24}), for all drugs, are corresponding with the theoretical daily permeated amount (Q_{24}).

Table 8: Relevant parameters estimated for the formulation of TTS

Drug	D_o (mg)	J (mg $cm^2\ h^{-1}$)	Q_{24} (mg)	PQ_{24} (mg)	D_{ss} (mg)	C_{ss} (mg)
BPAA*	600	0.155	15.16	16.30	12.40	12.56
Diclofenac	50	0.085	5.97	6.42	6.80	5.66
Diclofenac Na	75	0.032	3.58	3.85	2.56	4.81
Indomethacin	75	0.130	11.08	11.91	10.41	9.17
Piroxicam	20	0.041	6.81	7.32	3.20	3.22

*Biphenylacetic acid

Discussion

Non-steroidal anti-inflammatory drugs were selected basically on their physical and chemical properties, which were identified as important to transdermal delivery. In this pre-formulation studies, further physicochemical properties including ionization constant, aqueous and non-aqueous solubility and partition coefficient were established for the feasibility of NSAIDs formulation to systemic delivery. It is well known that molecular characteristics, such as size and shape, of a penetrant play an important part in penetration through the skin. An inverse relationship appears to exist between absorption rate and molecular weight where small molecules penetrate more rapidly than large ones [18]. Accordingly, an attempt was made to reduce the molecular weight of diclofenac sodium by changing the drug to a weak acid. IR and NMR spectrum confirm that the aim of converting diclofenac sodium to diclofenac is well established in which the substitution of sodium by hydrogen was successful. The pk_a values reported in literature are 3.9 for biphenylacetic acid [18], 5.3 for diclofenac sodium [10], 4.5 for indomethacin [19] and 6.3 for piroxicam [7]. Therefore, pK_a values obtained in the present study are in agreement with those previously reports. It has been reported that surface of skin is slightly acidic, *p*H 5.6, and lowers are at physiological *p*H 7.4 [20]. At the physiological *p*H 7.4, the percent ionized of NSAIDs studied was almost completely ionized. Thus, passage through the polar regions and aqueous filled pores in the biomembrane will be an important route of penetration. The melting points obtained in the present study are as reported [19]. Plots of the observed solubility versus melting points of drugs should have a linear relationship [21], this relationship was obtained in the present study. Except for diclofenac, higher solubility values were observed with the drugs that have lower melting points. This agrees with published relationships demonstrated using

different series of compounds [21]. With exception of diclofenac sodium that has slightly higher aqueous solubility, much better solubility was obtained from further studies in an aqueous phosphate buffer (*p*H 7.4). A higher solubility of drugs studied in *n*-octanol was obtained in comparison to their aqueous solubility. The behavior of drugs in lipid solvent is considered to be quite important in relation to transdermal penetration [22]. The *n*-octanol, generally, is the most appropriate non-polar phase and is consequently the most widely used for partition studies [23]. Dearden (24) suggested that the measurement of partition coefficient of many weak acids is only valid within a *p*H range of about three units above the pK_a. It has been criticized the assumption that the ionized species of an ionizable drug is insoluble in the lipid phases especially when *n*-octanol, previously saturated in water, is used as the small amount of water in the *n*-octanol layer might be expected to accommodate some concentration of the ionized species [25]. The difference in log *P* values between unionized and ionized species decreased steadily with increasing log *P* due to ion pair formation with buffer count-ions [25]. Ideally, log *P* value should be between 1 and 3 for drugs that may have the ability to penetrate the skin [26]. Since the log *P* of drugs studied are found in the range of 1.85-2.84 for *n*-octanol/water system in which highly lipophilic, their presence in the predominantly ionized form introduces the possibility of the excretion of the ionized form as ion pairs into the aqueous saturated *n*-octanol phase. Many homologous series showed more complex parabolic or bilinear relationship between pharmacological activity and log *P* value [27]. It seems that with higher values of log *P*, the compounds are so lipid soluble and they remain dissolved in the stratum corneum which will not readily pass into the water-rich viable tissue. The log *P* values obtained in the present study are of the same order as those of glyceryl trinitrate (log *P* = 2.05), chlordiazpoxide (log *P* = 2.5) and timolol (log *P* = 1.91) for which their systemic delivery by the transdermal route has been demonstrated [28]. The obtained stability data of NSAIDs studied confirms that ionic strength of the buffer solution which had been in contact with the dermis of mouse skin and that of the fresh phosphate buffer solution has no significant influence on the stability of NSAIDs in the solution. The skin permeation profiles show that drugs studied seem to have the ability to penetrate the layers of hairless mouse skin. Since the stratum corneum is a lipophilic membrane, a lipophilic drug is expected to be more permeable in such a layer than a drug with a lower lipophilicity and, on contrary, the hydrophilic drug may penetrate more easily than the lipophilic drug across the viable skin, which is known to be less lipophilic [29]. Indomethacin, BPAA and piroxicam (most lipophilic) were found to penetrate across the mouse intact skin much rapidly than diclofenac sodium and diclofenac (less lipophilic). Diclofenac was shown the highest *in vitro* permeation rate constant compared to other NSAIDs, however, it also has modest flux evaluated in the diffusion cell [30]. The lag time is the first obstacle to transdermal penetration which could be considered as the first limiting factor for TTS formulation and 10% of the exposure time of the TTS is considered reasonable upper value for the lag time [17, 31]. In the present study, the estimated mean values of lag time are higher than this limitation. However, it is interesting to see that lag time values for all drugs studied have the same order of magnitude for the intact and the stripped skin. The lag time for the intact skin is 2 - 4 fold greater than that for the stripped skin. Similar findings for other groups of drugs were obtained by other researchers [32, 33]. The steady-state rate permeation across the intact and stripped skin was found to be proportional to the solubility of drugs in the stratum corenum or in the viable skin, respectively. The findings indicate that the drug solubility in the skin is an important parameter for controlling the permeation rate of NSAIDs studied. The solubility in the viable skin and the diffusivity across the stratum corenum were observed to be dependent on the penetrant. The deviation of the solubility data is probably due to the drug binding in the stratum corenum [4]. The aim of establishing a relationship between physicochemical properties of the NSAIDs studied and their percutaneous penetration was to estimate the main penetration parameters and evaluate potential candidate for formulation of TTS. The molecular volume (V_m) is considered an important parameter for controlling the transport of drugs, especially lipophilic molecules, across the skin [34]. The combined effect of lipid solubility and size on diffusion through the skin has been studied for a number of compounds, including NSAIDs [34]. The present study demonstrated that an increase in the V_m of the drug results in an order of magnitude decrease in the predicted transdermal flux. According to Flynn and Steward [35], the limitation for the potential use of a drug as a TTS is the low permeability constant (K_p) value. It has been considered that K_p values obtained in hairless mouse skin could be nearly three times higher than the corresponding values obtained in humans [36]. The predicted K_p values in this study,

except for biphenylacetic acid and indomethacin, are of the same order of magnitude as those corresponding to clonidine (3.82 x 10^{-3} cm^2 h^{-1}), and nicotine (2.96 x 10^{-3} cm^2 h^{-1}) reported for human skin [37]. Indomethacin being the drug with the highest K_p mean value (5.70 x 10^{-3} cm^2 h^{-1}) and diclofenac sodium with the lowest K_p mean value (2.06 x10^{-3} cm^2 h^{-1}). The parabolic function was found to fit the K_p values obtained in the present study using the physicochemical parameter log *P*. The use of this relationship has been justified by the pathway that the drug must follow in order to reach the biphasic, a path which is constituted by multiple hydrophilic and lipophilic barriers that are involved in the transport of the drug [23]. The parabolic correction has also been used to predict the intestinal absorption of a distinct homologous series of compounds from their lipophilic parameters, particularly the partition coefficient [38, 39]. In fact, the relationship between transdermal permeability and partition coefficient of the NSAIDs studied follows the parabolic function, corroborating the utility of the proposed parabolic equation found, Log K_p = -5.242 + 1.757 (log P) + 0.26 (log P)^2. For a candidate to be formulated in a TTS design it must have a certain thermodynamic activity to guarantee sufficient drug penetration to reach steady-state conditions [40]. It is clear that free energy of a chemical reaction depends on the heat energy and entropy of the reactants and its concentration [30]. Thermodynamic activity is the driving force of the drug partitioning process into the skin in which the interfacial transport of a drug to the subcutaneous is promoted when thermodynamic activity increases, providing a higher permeation through the skin, indicatively, the thermodynamic activity of a drug gets higher when concentration is increased [41]. The candidate needs to achieve an adequate flux, and in this sense, a low solubility in the vehicle for a drug as this could be considered as an obstacle to its formulation in a TTS. Although the problem of extrapolation of animal data to humans is always speculative, the aim was to predict the permeation behavior of the drugs studied with the purpose of achieving an approximate estimation of theoretical steady-state plasma levels reached after TTS application. The physicochemical criteria (i.e. diffusion and partitioning), which determine the feasibility of NSAIDs studied for transdermal delivery, were identified. As predicted permeated amounts during 24 hours for all drugs correspond with the calculated amount, a prediction from other related effects including vehicle, *p*H, and penetration enhancers should be considered as they influence drug penetration from TTS [42].

Conclusion

The physicochemical properties including ionization constant, aqueous and non-aqueous solubility and partition coefficient of NSAIDs studied were identified as important to transdermal delivery. The parameters, in particular, lipid solubility, molecular volume and partition coefficient were correlated well with skin permeability. The relationships established provided a simple format for explaining the essential physical and chemical properties that govern transport of NSAIDs across hairless mouse skin.

Ethical issues

Including plagiarism, Informed Consent, data fabrication or falsification and double publication or submission have completely been observed by authors.

Author's contribution

MB Lambert and PB Deasy have conceived, designed and supervised the study. AAM Amara has performed the research, collected/analyzed the data, and drafted the manuscript. All authors equally revised the manuscript for important intellectual context and approved the final manuscript.

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Supplementary

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