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Percutaneous absorption of ibuprofen: Vehicle effects on transport through rat skin

W.J. Irwin, F.D. Sanderson* and A. Li Wan Po+

Drug Development Research Group, Pharmaceutical Sciences Institute, Aston University, Aston Triangle, Birmingham, B4 7ET, (U.K.)

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Summary

The transport of ibuprofen from propylene glycol vehicles through rat skin has been monitored in vitro. Skin stored for up to 4 months showed no significant change in its permeability to the drug but when the same tissue was used for consecutive experiments dramatic increases in flux were observed. Significant effects on drug permeation caused by vehicle composition and pH were found and solubilities and vehicle-skin partition coefficients were measured for all vehicles. As the concentration of propylene glycol increased permeation was related to the increase in solubility and reduction in partition coefficient. The pH effect was related to the degree of ionisation of the drug and to the effective concentration or degree of saturation when solutions were used. With suspensions of ibuprofen penetration rates were effectively independent of pH until high values were used when the vehicle interfered with the barrier properties of the skin.

Introduction

Vehicle effects may have a profound influence upon the percutaneous delivery from topical products. In the case of ionisable molecules pH and ionpairing agents, in addition to the proportion of organic cosolvent and the presence of specific enhancers, may modify the transport profile. Although, in general, ionisation reduces topical availability (Flynn, 1989) there are reports where the reverse is the case. For example, salicylic acid delivery has been claimed to be elevated with quite moderate pH increases (Loftsson, 1985). Additionally, cosolvents may alter the barrier properties of the skin (Poulsen, 1972) and there may be concentration- and time-dependent effects. In view of the reports on both in vitro and in vivo percutaneous absorption of ibuprofen (Akhter and Barry, 1985; Katz et. al., 1986; Berner and Wagener, 1987; Ito et al., 1988) and the recognition of the importance of ionisation processes in topical delivery (Oakley and Swarbrick, 1987) we have studied these interactions by determining the vehicle-skin partition coefficients and solubilities of ibuprofen in a series of aqueous propylene glycol systems with different cosolvent compositions

Correspondence: W.J. Irwin, Drug Development Research Group, Pharmaceutical Sciences Institute, Aston University, Aston Triangle, Birmingham B4 7ET, U.K.
**Present address:* SmithKline-Beecham, Research Division, Clarendon Road, Worthing, West Sussex BN14 8QH, U.K.
+Present address: Department of Pharmacy, The Queen's University of Belfast, 97, Lisburn Road, Belfast BT9 7BL, U.K.

or pH values. These have been related to the steady-state flux of the drug determined in the same vehicle using in vitro diffusion through isolated rat skin.

Experimental

Apparatus

HPLC analyses were undertaken using a system constructed from an Altex 100A dual-piston reciprocating solvent-metering pump and a reversed-phase stainless steel Shandon-type column (10 cm × 4.6 mm i.d.) packed with Hypersil-ODS (5 μm). Samples were introduced by means of a Rheodyne 7125 injection valve, fitted with a 20 μl loop, and detection was accomplished with a Pye LC3 variable wavelength UV detector, fitted with an 8 μl flow cell, and operated at a wavelength of 225 nm with a sensitivity of 0.01 AUFS. The mobile phase consisted of aqueous acetonitrile (55%), adjusted to pH=2.0 with phosphoric acid (0.45%), and delivered at 1 ml min^{-1}. Maximum concentrations of 45 μmol l^{-1} (9.3 μg l^{-1}) were used and calcium fenoprofen 1.1 μmol l^{-1} (0.6 μg ml^{-1}) in 50% aqueous methanol was used as the internal standard. In general, 1 ml of the test solution was treated with 4 ml of the internal standard solution and 10 μl were injected into the HPLC.

Diffusion experiments were undertaken in a glass diffusion cell of the Franz-type (Franz, 1975) with a wide-necked, upper donor compartment. The lower, receiver chamber had a capacity of 20–30 ml and was jacketed, to enable the use of a circulating water bath (Churchill) to maintain the temperature at 37°C. A teflon-coated stirrer provided constant agitation of the receiver solution and a side arm allowed the withdrawal of samples from this compartment. The cells were assembled with the excised skin sandwiched, epidermis uppermost, between the flat ground glass surfaces of the donor and receiver chambers, a stainless steel mesh in the lower chamber provided support. The two cell halves were sealed with PTFE tape and Nescofilm, secured with a spring clamp and the donor chamber was fitted with a perspex lid to prevent evaporation. The available area for diffusion between the compartments was about 2.0 cm^2. The receiver solution was prepared containing equal volumes of propylene glycol and McIlvaine buffer pH 5.0 double strength (Elving et al., 1956) with a final pH of 5.5. A volume of 20–30 ml was allowed to equilibrate overnight with the skin in the diffusion cells. The solution was replaced the following morning prior to adding the appropriate donor solution (5 ml). Six cells were set up simultaneously and at one hourly intervals samples (1 ml) were withdrawn from the receiver for HPLC analysis, and replaced with a fresh aliquot of receiver solution.

Materials

Preparation of rat skin. The membrane used was full thickness skin section from the abdominal surface of male Wistar rats. Hair was removed with clippers and rectangular sections of skin, several centimetres square were excised from the animal, and adhering fat and other visceral debris were removed. The membrane was then either used immediately or was stored in a freezer at −18°C between sheets of aluminium foil. Samples free of stratum corneum were also shaved with a twin blade razor and were stripped 30 times with sellotape prior to its removal from the animal. Skin layers were isolated by soaking skin in 2 M sodium bromide solution (Walker and Scott, 1984) for 2 h and then gently rubbing to remove the epidermis. The dermis was used immediately in one experiment and was frozen overnight prior to use in another.

Permeation studies. The donor solution was a suspension of ibuprofen (5 ml) in 50% propylene glycol (pH 5.5). Samples (1 ml) were taken from the receiver compartment at 1 h intervals for a period of 12 h and these were analysed by HPLC. To study the effect of storage, experiments with ibuprofen were performed using skin stored for periods of 20, 80, and 120 days. The effect of repeated dosing was monitored by performing consecutive experiments with the same membrane. Between dosings compartments were thoroughly washed and then left overnight in contact with 50% propylene glycol (pH 5.5). The system was then rewashed before the addition of a fresh suspension of ibuprofen (5 ml). This procedure was repeated for a total of three dosings. A similar experiment was

performed using aqueous McIlvaine buffer (pH 5.5) instead of 50% propylene glycol (pH 5.5) as the donor vehicle and receiver phase.

To monitor the effect of propylene glycol concentration, suspensions of ibuprofen in solutions of propylene glycol (0–60%) in McIlvaine buffer, adjusted to pH 5.5, were prepared (Sanderson, 1986). The receiver phase in each case was the corresponding aqueous propylene glycol buffer. Suspensions (pH 3.47–9.02) and solutions (pH 3.47–7.2) in aqueous McIlvaine buffer containing 50% propylene glycol were also prepared. The solutions contained 140 μg ml^{-1} and sampling was over a period of days.

Solubility determinations. Excess ibuprofen was added to aliquots of the drug-free vehicle and was agitated at 37°C in a shaking water bath for 2 days. The solutions were filtered, diluted and analysed by HPLC.

Determination of partition coefficients. The 50% propylene glycol (pH 5.5) vehicle was presaturated overnight with octanol in a water bath at 25°C and solutions of ibuprofen (70 mg in 250 ml) in this vehicle were prepared. Aliquots (40 ml) of this solution were shaken with 5 ml aliquots of the presaturated octanol and the drug content was determined in both phases by HPLC. Stock solutions of ibuprofen (120 μg ml^{-1}) were prepared in the vehicles of varying pH or propylene glycol content and these were diluted 1 in 20 with the appropriate drug free vehicle. Aliquots (10 ml) were added to vials containing 0.35 g of whole rat skin and to two empty vials to act as controls. The solutions were allowed to equilibrate for 4 weeks and the solutions analysed by HPLC. The density of the vehicle was determined using a 25 ml density bottle, so that weight ratios instead of volume ratios could be used in partition coefficient determination. The solutions were analysed by HPLC.

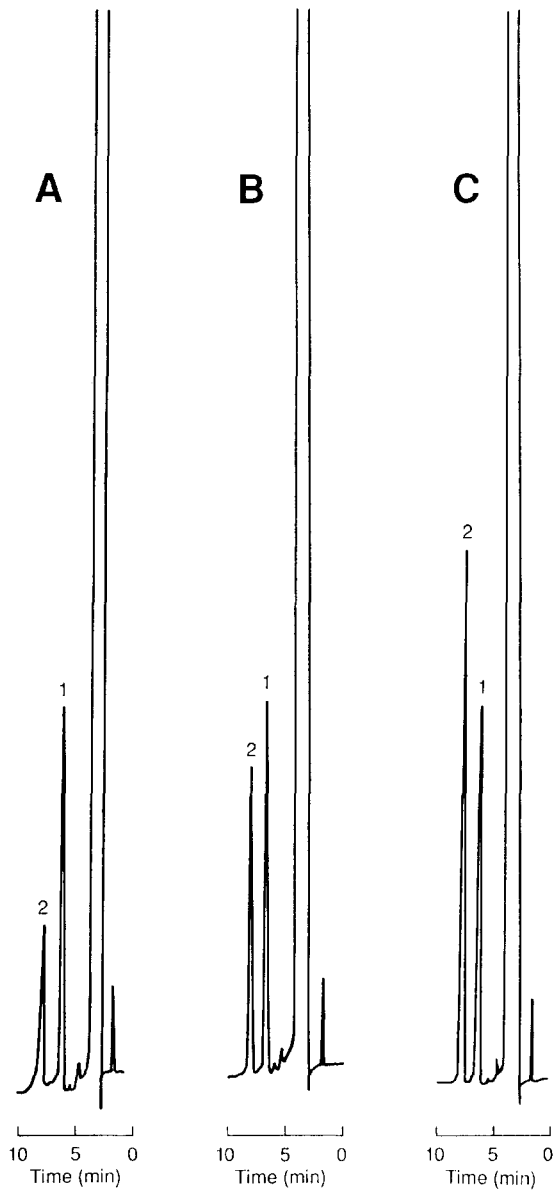

Fig. 1. HPLC chromatograms of the permeation of ibuprofen through rat skin from a suspension in 50% aqueous propylene glycol (pH 5.5): (1) fenoprofen; (2) ibuprofen.

Results and Discussion

The permeation of ibuprofen through rat skin monitored by HPLC is shown in Fig.1 and the results of using samples stored for various periods up to 4 months are listed in Table 1. No significant change in the permeability properties of the skin occurred during this time but skin samples were generally used within 7 days. Little change in the permeability of human skin to radiolabelled water over a period of about one year has also been demonstrated (Harrison et al., 1984).

Profiles reveal substantial variation between

TABLE 1

Effect of storage on the permeation of ibuprofen through rat skin [values in parentheses are standard errors]

Storage (days)	Flux($\times 10^3$) (μmol cm^{-2} h^{-1})	Lag time (h)	K_p($\times 10^3$) (cm h^{-1})	n
0	59.67 (7.03)	5.71 (0.29)	3.99 (0.47)	11
20	60.69 (9.46)	4.76 (0.44)	4.06 (0.63)	6
80	68.73 (7.66)	4.03 (0.20)	4.60 (0.51)	6
120	77.85 (12.09)	4.30 (0.15)	5.21 (0.81)	6

replicate runs (Southwell et al., 1984) but calibration with an internal standard is compromised as different vehicles can affect the permeation of drugs to differing extents. An alternative approach to standardisation is to measure the flux of radiolabelled water through hairless mouse (Matoltsy et al., 1968) or human skin (Dugard and Scott, 1986) prior to use. In contrast to the storage results, in this study repeated use of the same membrane yielded significant enhancement of permeability. Table 2 records flux data for a totally aqueous vehicle and one containing 50% propylene glycol, both adjusted to pH 5.5. All profiles reveal a lag time, a period of steady-state flux and substantial variability. Both systems showed a rapid increase in permeation with the second treatment followed by a smaller increase with the third. The repeated use of the aqueous vehicle completely removed the lag time on day 3, possibly due to hydration of the skin and high retention of ibuprofen. These observations suggested that variability was not readily reduced by standardisation and subsequent experiments were repeated five or six times, where possible, to provide average permeation profiles.

The effect of propylene glycol concentration on the steady state flux of ibuprofen from suspensions was studied with vehicles containing 0 to 60% propylene glycol. Results are recorded in Table 3.

The steady-state flux of drug (J) through the rat skin (thickness, h) may be modelled by:

$$J = P_m D \Delta C_s / h \quad (1)$$

where P_m is the membrane-vehicle partition coefficient, D is the diffusion coefficient of the drug in the membrane and ΔC_s is the solute concentration difference across the membrane. The permeability

TABLE 2

Effect of storage on the permeation of ibuprofen from suspension through rat skin [values in parentheses are standard errors]

Treatment (days)	Flux($\times 10^3$) (μmol cm^{-2} h^{-1})	Lag time (h)	K_p($\times 10^3$) (cm h^{-1})	n
Propylene glycol (50%, pH 5.5)				
1	72.52 (11.4)	3.43 (0.09)	4.85 (0.76)	4
2	126.42 (9.4)	2.42 (0.11)	8.45 (0.63)	4
3	146.15 (6.1)	2.43 (0.21)	9.77 (0.41)	4
Aqueous buffer (pH 5.5)				
1	24.67 (5.4)	3.20 (0.12)	11.03 (2.42)	5
2	59.43 (6.2)	0.94 (0.38)	26.58 (2.77)	5
3	67.77 (5.3)	–	30.30 (2.38)	5

constant is defined as $K_p = P_m D/h$. In an ideal system h and D are assumed constant and ΔC_s approximates to the vehicle concentration of the drug (C_v) and hence $J \propto C_v P_m$. Additionally, as the solubility of the drug in the vehicle increases the partition into the membrane falls. The product $C_v P_m$ is essentially constant and a uniform flux is expected (Poulsen, 1972) if the vehicle exerts no effect upon the skin. In contrast, the flux data held in Table 3 show essentially a steady-state flux which increases progressively with propylene glycol concentration. The permeability constant, however, peaks at about 20% propylene glycol (Table 3) where the efficiency of drug delivery into the skin from the saturated solution appears to be maximal. The results suggest that the vehicle may be modifying the partition properties of the membrane and Table 4 presents data for the partition

TABLE 3

Effect of propylene glycol concentration on the permeation of ibuprofen from suspension through rat skin [values in parentheses are standard errors]

Propylene glycol (%)	Flux($\times 10^3$) (μmol cm^{-2} h^{-1})	Lag time (h)	K_p($\times 10^3$) (cm h^{-1})	n
0	25.21 (5.92)	3.24 (0.12)	12.28 (2.65)	5
10	33.25 (7.08)	4.56 (0.40)	13.32 (2.84)	4
20	79.21 (7.76)	3.71 (0.42)	25.06 (2.46)	6
30	51.19 (5.96)	4.06 (0.61)	11.87 (1.38)	6
40	59.28 (5.48)	4.00 (0.99)	7.33 (0.68)	6
50	70.09 (4.41)	3.73 (0.87)	4.69 (0.30)	15
60	100.10 (32.72)	4.74 (0.46)	2.55 (0.83)	6

of ibuprofen between whole skin and various concentrations of aqueous propylene glycol (pH 5.5), together with solubilities of the drug in the vehicles.

As expected, an increase in solubility with a corresponding reduction in partition coefficient was observed as the volume fraction of propylene glycol increases from 30 to 60%. At levels of 20–30% propylene glycol there appears to be a maximum partition coefficient, probably due to penetration of propylene glycol and an enhanced solubility of ibuprofen in the skin. The product of the partition coefficient and vehicle solubility (C_vP_m) are also listed in Table 4 and predict a trend of increasing flux as propylene glycol content increases. Although broad error limits are apparent in this system the overall flux is dependent upon C_vP_m ($J \times 10^3 = 3.417\, C_vP_m + 17.942$, r=0.84) while K_p, which also depends upon the diffusion coefficient, shows a much weaker correlation (r=−0.548).

An extended method of data analysis (Turi et al., 1979) assumes membrane thickness to be constant and that changes in steady state flux are due to variations in the product of the remaining three parameters ($J \propto C_vP_mD$). Drug solubility is exponentially related to the volume fraction of cosolvent (f) by:

$$C_v = C_w \cdot \exp(\alpha f) \quad (2)$$

where C_v is the solute solubility in a mixed binary aqueous solvent with volume fraction f of a non-aqueous cosolvent and C_w is the solubility in water. The constant α is characteristic of the system. The partition coefficient of a drug (P_m) is equal to its solubility in the membrane (C_m) divided by its solubility in the binary solvent ($P_m = C_m/C_v$) which leads to:

$$P_m = P_w \cdot \exp(-\alpha f) \quad (3)$$

where P_w is the partition coefficient relative to water. Substitution of these expressions for C_f (C_v) and P_f (P_m) into Eqn 1 leads to:

$$J = DC_w \cdot \exp(\alpha f) \cdot P_w \cdot \exp(-\alpha f)/h \quad (4)$$

and

$$J = DC_wP_w/h \quad (5)$$

This mirrors the earlier treatment which predicts that steady state flux of a drug through the skin should be constant for saturated binary solvents which do not have a vehicle effect on the skin. If the solvent blend influences the drug solubility in the skin a vehicle effect will be observed and the partition coefficient is expressed as:

$$P_m = P_w \cdot \exp(-\beta f) \quad (6)$$

where β is a constant different in magnitude to α. Eqn 4 now becomes:

$$J = DC_w \cdot \exp(\alpha f) \cdot P_w \cdot \exp(-\beta f)/h \quad (7)$$

and the steady state flux of the drug through skin will depend upon the particular values of the constants α and β.

Plots of natural logarithm solubility and partition coefficient against volume fraction of propylene glycol (ln transforms of Eqns 2 and 3) are bi-

TABLE 4

Solubility, partition coefficients into rat skin and C_vP_m values for ibuprofen in aqueous propylene glycol at pH 5.5 [values in parentheses are standard errors]

Propylene glycol (%)	Solubility C_v (mg ml^{-1})	P_m (dry)	P_m (wet)	C_vP_m (dry)
0	0.461 (0.023)	6.08 (0.73)	6.58 (0.60)	2.80
10	0.515 (0.022)	12.91 (0.44)	14.38 (0.30)	6.65
20	0.652 (0.015)	15.87 (1.04)	15.14 (1.28)	10.35
30	0.890 (0.007)	16.00 (0.56)	14.38 (0.33)	14.24
40	1.669 (0.026)	9.42 (0.43)	10.87 (0.69)	15.72
50	3.085 (0.196)	4.33 (0.21)	4.46 (0.18)	13.36
60	8.115 (0.697)	2.78 (0.24)	3.20 (0.30)	22.56

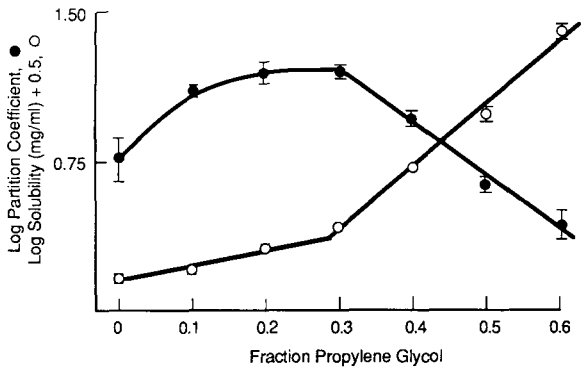

Fig. 2. Logarithm of vehicle-skin partition coefficient and of the vehicle solubility of ibuprofen as a function of propylene glycol concentration.

phasic (Fig. 2) with a linear relationship at higher concentrations of propylene glycol, which over the range 30–60% propylene glycol, yielded:

$$\ln C_v = -2.356 + 7.245f \qquad r = 0.993$$

and

$$\ln P_m = 4.588 - 6.028f \qquad r = 0.995$$

from which α=7.245 and β=6.028. Whenever α is greater than β the rate of permeation increases with the fraction of cosolvent. Over this range a mean value for D/h can be calculated ($D/h = K_p/P_m$) as 0.8766 cm h^{-1}. The intercepts yield notional values for C_w and P_w and values for the flux may be estimated from Eqn 7.

The effects of vehicle pH were investigated initially with equimolar buffered solutions of ibuprofen (140 μg ml^{-1}, 0.6 mmol l^{-1}) each containing 50% propylene glycol (pH 3.47–7.20). Data are recorded in Table 5.

It is apparent that as the pH is increased the steady state flux falls substantially accompanied by the expected large increase in the solubility of the drug. A plot of flux against the fraction of drug in the unionised form revealed a direct linear dependence. A pK_a value of 5.2 for ibuprofen was used in these calculations (Davis, 1975; Herzfeldt and Kummel, 1983). If the total flux observed (J_{obs}) is dependent upon the flux of ionised (J_i) and unionised (J_u) species and the fraction of drug in each state (α, 1–α) one can write:

$$J_{obs} = \alpha J_i + (1-\alpha)J_u$$

which may be linearised to $J_{obs}/\alpha = J_i + (1-\alpha)J_u/\alpha$. Thus, a plot of J_{obs}/α against $(1-\alpha)/\alpha$ enables the flux of the ionised and unionised species to be estimated as:

$$J_{obs}/\alpha = 2.53\times10^{-3}(1-\alpha)/\alpha - 5.70\times10^{-5} \qquad r = 0.999$$

The small, negative intercept suggests that, in accordance with the pH-partition hypothesis, the ionised species does not penetrate the membrane to any significant extent and that when the drug is totally ionised the permeation rate should be negligible, as is apparent in this case.

Table 6 lists both wet and dry partition coefficients. The variability of those based upon wet weights is due to vehicle effects. Weighing the skin before and after the partition experiments shows that the weight is more than doubled at pH 3.47 and increases by some 40% at pH 7.20. Other values are unaffected. Modification of skin keratin, which has an isoelectric point between 3.7 and 4.5, at low pH values may alter skin hydration (Barry,

TABLE 5

Effect of pH on the permeation of ibuprofen from solution through rat skin [values in parentheses are standard errors]

pH	Flux($\times10^3$) (μmol cm^{-2} h^{-1})	Lag time (h)	K_p($\times10^3$) (cm h^{-1})	Solubility (μmol ml^{-1})	Saturation (%)	Unionised (%)	n
3.47	2.482 (0.303)	11.82 (2.85)	4.313 (0.527)	3.905	14.73	98.2	5
4.85	1.774 (0.154)	24.53 (5.97)	3.274 (0.284)	8.168	6.63	69.1	4
5.17	1.023 (0.137)	18.95 (3.77)	1.991 (0.266)	9.452	5.44	51.7	6
5.55	0.795 (0.113)	7.32 (0.61)	1.470 (0.209)	14.955	3.62	30.9	6
7.20	0.227 (0.071)	60.65 (5.29)	0.339 (0.106)	78.879	0.0848	1.0	6

TABLE 6

Effect of pH on solubility, partition coefficients into rat skin and C_vP_m values for ibuprofen in 50% aqueous propylene glycol [values in parentheses are standard errors]

pH (%)	Solubility C_v (mg ml^{-1})	P_m (dry)	P_m (wet)	C_vP_m (dry)
3.47	0.806 (0.063)	8.60 (0.74)	3.65 (0.26)	6.93
4.85	1.685 (0.022)	8.03 (0.89)	9.29 (0.83)	13.53
5.17	1.950 (0.098)	6.93 (0.07)	8.05 (0.37)	13.51
5.55	3.805 (0.196)	4.33 (0.21)	4.46 (0.18)	16.48
7.20	16.479 (0.026)	1.03 (0.18)	0.75 (0.13)	16.97

1983), while at higher pH values extraction of water binding materials and dissolution of keratin may occur. The pH-dependent partition of ionisable molecules may be modelled by:

$$P_{obs}/\alpha = P_i + (1-\alpha)P_u/\alpha$$

where P_{obs} are the observed partition coefficients at various fractions ionised (α) and P_i, P_u are the partition coefficients of the ionised and unionised species respectively (Irwin and Li Wan Po, 1979). Using the dry weight partition data, and plotting P_{obs}/α against $(1-\alpha)/\alpha$ the partition coefficient of the unionised and ionised forms of the drug can be calculated:

$$P_{obs}/\alpha = 8.69(1-\alpha)/\alpha + 3.70$$

The partition coefficient of the unionised form is about 8.7 and that of the ionised form of ibuprofen is 3.7, somewhat higher than the practically determined value.

Although the pH of the vehicle influences skin hydration, little deviation from the predicted linear dependence of steady state flux on fraction unionised is revealed. Since equimolar solutions were used for this study the degree of saturation is reduced as the pH increases (Fig. 3). The steady-state flux (J_{obs}) is dependent upon the fraction of saturation (F_s) according to $J = 0.0161F_s + 2.51\times10^{-4}$ (r=0.956) which predicts that the steady-state flux for a saturated solution will be 0.0161 μmol cm^{-2} h^{-1}. To reduce the effect of this phenomenon permeation rates from suspensions were examined and results are recorded in Table 7.

Between pH 3.47 and 7.20, using suspensions of ibuprofen, no significant effect on the rate of permeation was noted. However, the permeability constant appears somewhat larger at lower pH values. Provided the vehicle does not affect the skin and a saturated solution is used, the steady state flux is essentially constant. The average flux for the suspensions was 0.095 μmol cm^{-2} h^{-1} some 6-fold higher than predicted from the solution data. It is possible that the flux drops off more quickly from solutions as the donor phase concentration is

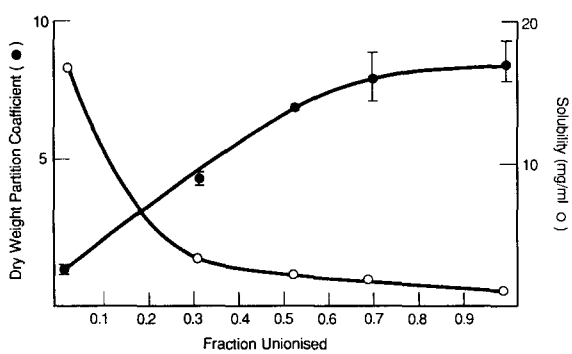

Fig. 3. Dry weight vehicle-skin partition coefficient and the vehicle solubility of ibuprofen as a function of fraction ionised.

TABLE 7

Effect of pH on the permeation of ibuprofen from suspension in 50% aqueous propylene glycol through rat skin [values in parentheses are standard errors]

pH	Flux($\times10^3$) (μmol cm^{-2} h^{-1})	Lag time (h)	K_p($\times10^3$) (cm h^{-1})	n
3.47	82.36 (8.10)	4.50 (0.27)	21.094 (2.075)	6
4.85	99.56 (15.22)	4.17 (0.36)	12.189 (1.863)	6
5.17	113.43 (21.47)	3.77 (0.15)	12.000 (2.271)	5
5.55	70.09 (4.41)	3.73 (0.23)	4.687 (0.295)	15
7.20	109.84 (23.07)	4.52 (0.25)	1.375 (0.289)	6
8.16	199.37 (33.69)	3.05 (0.28)	4.645 (0.785)	6
9.02	491.18 (101.99)	2.20 (0.26)	9.427 (1.958)	5

continually depleted and since data were collected only up to a maximum of 15% saturation, it is difficult to extrapolate to the fully saturated solution.

The two additional systems of higher pH, 8.16 and 9.02, showed 2-fold and 5-fold increases in the permeation rate respectively compared with the mean of the other values. This increase in flux is accompanied by a significant reduction in lag time (Table 7) and suggests that the alkaline pH values initiated adverse effect on the skin barrier, thereby reducing its effectiveness.

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