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Ionisation and the effect of absorption enhancers on transport of salicylic acid through silastic rubber and human skin

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Abstract

Propose: to investigate if salicylic acid (SA)-permeation through excised human skin (HS) and silastic rubber (SR) conforms to the pH-hypothesis and to assess the influence of a range of absorption enhancers on the transport of SA with and without a transmembrane pH-gradient. *Methods:* Franz cells were used to study SA permeation from solutions and saturated suspensions. McIlvaine buffers were used to maintain transmembrane pH-gradients. Membrane pretreatment was used to study the action of absorption enhancers. *Results:* the flux of SA from solutions was dependent upon the vehicle pH and permeant concentration was directly related to the degree of ionisation of the solute. Flux from suspensions was independent of pH, since the level of unionised drug, the predominant diffusing species, was maintained at the intrinsic saturated solubility at all pH values. The observed SA flux enhancement across human skin without a transmembrane pH-gradient was not significantly different from the enhancement with a pH-gradient for all of the absorption enhances used, except for dodecylamine. *Conclusions:* the results showed that SA permeation conformed to the pH-partition hypothesis. The evidence from absorption-enhancer pretreatment demonstrated that, under certain conditions, the transdermal penetration enhancement of a number of topical enhancing compounds, including Azone and oleic acid can be explained without recourse to ion-pair phenomena.

Keywords: Percutaneous absorption; Ionisation; Salicylic acid; Enhancers; pH-partition hypothesis

1. Introduction

The physicochemical properties of a vehicle and drug have a direct influence upon the drug release rate from topical preparations. The transdermal delivery of topically applied drug's is dependent on both this release rate and the drug's permeability through the skin. According to the pH-parti-

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tion hypothesis, only the unionised forms of drugs are able to pass through lipoidal membranes. Early absorption studies on salicylates (Arita et al., 1970; Siddiqi and Ritschel, 1972), lignocaine (Menczel and Goldberg, 1978) and carboxamine (Arita et al., 1970), have supported this hypothesis. However, there has been increasing evidence that the ionised species can contribute to transdermal absorption. Evidence has been provided (Wallace et al., 1978) for parallel penetration pathways and shunt routes in the stratum corneum. These are involved in the penetration of ionised drug species. To support this, increasing the vehicles pH increases the solubility, ionisation and steady-state penetration of methotrexate (Vaidyanathan et al., 1985). Ionised compounds may also penetrate biological membranes through the hydrated keratin matrix (Flynn, 1985) or aqueous regions (Oakley and Swarbrick, 1986, 1987). This is in contrast to unionised drugs which principally penetrate *via* the lipid regions. However, when the penetrating species exists in both ionised and unionised forms, it is the unionised species that penetrates most rapidly through the skin (Touitou and Donbrow, 1982; Swarbrick et al., 1984).

Santi et al. (1991) found that nicotine did not follow the pH-partition hypothesis when partitioned between isopropyl myristate (IPM) and McIlvaine buffers, although verapamil did. This was in contrast to Oakley and Swarbrick (1987) who found that nicotine followed the pH-partition hypothesis with IPM, but formed ion-pairs and deviated from the hypothesis with a number of other organic compounds. It was suggested that this discrepancy was due to the different buffers employed in the two studies. A study using proxicromil (Lee et al., 1985), a compound whose ionic species readily forms ion-pairs with simple counter ions, showed that it has the ability to permeate biological membranes at physiological pHs that are well removed from its pK_a of 1.93 ± 0.06 (Swarbrick et al., 1984). The possibility of ion-pair formation of *m*-azidopyrimethamine with an anion present in the skin membrane was stated as the reason for the contribution of the ionised species to the observed partition coefficient into hairless mouse skin (Baker et al., 1990). From the wide range of results and interpretations of the transport of ionised compounds across absorption barriers, the in vitro diffusion of compounds can be significantly influenced not only by the physicochemical properties of the vehicle and the drug, but also by experimental parameters. The membrane type, the receiver-phase composition and the vehicle have significant effects upon the permeation profiles of methyl salicylate (Walkow and McGinity, 1987a) and SA (Walkow and McGinity, 1987b) Lee et al., (1987) found that the ionic species of sodium salicylate in aqueous media did not permeate through hydrophobic silicone rubber, which was in contrast to sodium salicylate in non-aqueous media, such as ethanol and dioxane, where the formation of ion-pairs enabled significant permeation.

The penetration of SA from solutions and saturated suspensions through excised human epidermis (HS) and silastic membrane (SR) was investigated. The influence of pH, a transmembrane pH-gradient, donor pH, receiver pH and absorption enhancers upon skin penetration were studied to correlate these parameters to the drug-vehicle-membrane inter-relationships governing penetration. The first objective of this study was to assess the effects of ionisation upon the transport of SA across artificial and biological membranes using a buffer of similar pH in both the receiver and donor phase. This enabled comparison with work using pH-gradients and assess whether permeation is due to unionised species alone, as predicted by the pH-partition hypothesis, or if ionised species have a significant role to play in transdermal permeation. The experimental protocols are based on work by (Loftsson, 1985) and are designed to test his hypothesis that, under certain conditions, increased ionisation of SA can promote topical delivery. The second objective was to study the effects and relative enhancement ratios of putative enhancers upon the model permeant, SA, with and without a pH-gradient across the membrane. The purpose was to determine whether, in some cases, the mechanism of enhancement of absorption enhancers can be explained by partition effects alone without recourse to ion-pair mechanisms.

2. Experimental

2.1. Materials

Reagents used were sodium salicylate (BDH) and SA (BDH), and HPLC solvents were of Hypersolv grade. Enhancers used were lauric acid *N*,*N*-dimethylamide (LDA) (Sigma), caproic acid *N*,*N*-dimethylamide (CDA) (Sigma), dodecylamine (DCA) (BDH), Azone® (Nelson Research, a gift), oleic acid (OA) (Merck) and Transcutol®(TC) (Gattefossé, a gift). All the enhancers were presented as 0.5 M solutions in propylene glycol (PG) (BDH) or isopropyl myristate (IPM) (Croda). Whole skin samples were obtained from human legs following amputation and the abdominal region of human cadavers at post mortem. Separation of the skin layers was achieved by submerging whole skin in water at 60°C for 60 s and the epidermis was gently teased away from the dermis using blunt forceps and floated on water at room temperature (Kligman and Christophers, 1963). Silicone rubber membrane (Silastic® Medical Grade, 500-5, Dow Corning 0.005 in. thick) was cut into circular sections of approximately 2 cm in diameter and thoroughly rinsed in distilled water prior to use to remove the surface powder. The aqueous buffer solutions and assay of saliylic acid were as described earlier (Irwin and Smith, 1991).

2.2. Donor solution

SA solutions were prepared by dissolving the appropriate quantity of sodium salicylate in McIlvaine's buffer (231.8 mg in 100 ml; pH range 2–5.4), preserved with phenylmercuric nitrate (0.002%). Any pH adjustment required was performed using 2 M sodium hydroxide or 2 M hydrochloric acid. Saturated suspensions were prepared by dissolving an excess of SA in hot McIlvaine's buffer which was then allowed to cool in a water bath at 32 or 37°C. Further pH adjustment was performed using a 30% sodium hydroxide solution or 7 M hydrochloric acid.

2.3. Permeation procedure for ionisation studies

Jacketed Franz-type diffusion cells were used throughout the study (Franz, 1975) and the diffusion barrier (epidermis or silastic) was mounted between the two chambers and secured with a spring-clamp. The ground glass surfaces of the cell that came into contact with the membrane were smeared with high vacuum silicone grease (BDH Prod 33135) to prevent leakage. Receiver solutions were was maintained at 37°C by means of a water jacket and a thermostatically controlled water-circulator. The donor cell and the sample were sealed to minimise evaporation. The receptor chamber was filled with a McIlvaine buffer of the appropriate pH. The receptor volume was approximately 30 ml, which was determined accurately by weight. Diffusional barriers were left to equilibrate with the receptor solution overnight at room temperature. The following morning, the cell was warmed to 37°C and the receptor solution allowed to equilibrate. An aliquot of 2 ml of the formulation under study was introduced into the donor compartment. For analysis via HPLC, 70 μl samples were removed from the receptor compartment at regular time intervals during a total experiment duration of 420 min. After the withdrawal of each sample, the receptor fluid was replenished with an aliquot of the drug-free vehicle and the cumulative mass of drug transported calculated (Baker et al., 1990). The cumulative amount permeated per unit area was plotted against time and the linear section of the graph taken as the steady-state flux. Lag-time was estimated by extrapolation from this line. The observed permeability coefficient (k_{obs}) was calculated by dividing the flux by the solubility of the SA in the test vehicle for suspensions (Smith, 1997) and by the concentration of the permeate in the test vehicles for solutions.

2.4. Permeation procedure for enhancer studies

Assay procedures, donor solutions, solubility determinations and jacketed Franz-type diffusion cells were set up as described for the ionisation studies, except that the receptor cell was maintained at 32°C. Samples of the receptor phase (1

ml) were removed at appropriate time intervals over 15–420 min and were assayed by HPLC following suitable dilution as described earlier. Receiver solutions of pH 3.40 buffer were diluted with distilled water and pH 7.22 receiver solutions were diluted with 0.1 M HCl. Diluting the pH 7.22 buffer with 0.1 M HCl quenched the chromatographic interference induced by the relatively high pH of this buffer. Following assembly of the permeation cells, pretreatment with enhancers was effected by applying 1 ml of the enchancing solution to the membrane surface for 12 h at room temperature. After this period, the cells were warmed to 32°C and the pretreatment solution carefully removed. The membrane surface was then rinsed a number of times with distilled water. Excess water was removed and the membrane surface dabbed dry with soft tissue. Following this procedure, 2 ml of the saturated suspension of SA was then applied to the membrane surface. Six Franz-type cells were used for each experimental run. Three of these cells were pretreated with enhancer and the remaining cells remained untreated as controls. Unless otherwise stated, permeation profiles with a pH-gradient utilised a saturated SA suspension at pH 4.04 as a donor and a McIlvaine buffer at pH 7.2 as a receiver. Profiles without a pH-gradient used a similar SA suspension at pH 4.04 as a donor and a McIlvaine buffer at pH 3.4 as a receiver. Values of gradients, intercepts and means are presented plus and minus the standard error (S.E.).

3. Results and discussion

3.1. Permeation of salicylic acid from solutions without a pH-gradient

The effects of pH were initially investigated using equimolar donor solutions of SA (2 mg ml^{-1}, 14.48 mM) buffered to a pH range 0.44–5.13. Receiver solutions were McIlvaine buffers adjusted to a pH similar to that of the donor. The steady-state fluxes, permeability coefficients and lag times derived from solution permeation profiles are summarised in Table 1 for SR and Table 2 of HS. Observed permeability coefficients and steady-state flux increase with decreasing pH, whereas the lag times generally increase with increasing pH. The lag times for SA permeation through HS were somewhat longer than the through SR, although the difference was not statistically significant ($P = 0.105$). This contrasts with the steady-state flux which was greater for SR. The comparative fluxes over the pH range 2.27–5.13 were on average 1.60 times greater through SR than HS. Plots of steady-state flux

Table 1
Permeation data for salicylic acid across silastic rubber from 14.48 mM solutions[a]

pH	Flux (μmol cm^{-2} h^{-1})	K_{obs} (cm h^{-1})	Lag time (min)	*n*
0.44	1.31 (6.5×10^{-2})	9.0×10^{-2} (4.5×10^{-3})	0.97 (2.86)	2
2.27	0.97 (2.5×10^{-2})	6.7×10^{-2} (1.7×10^{-3})	2.50 (1.51)	2
2.72	0.72 (1.3×10^{-2})	5.0×10^{-2} (9.0×10^{-4})	2.38 (1.04)	2
3.13	0.55 (4.4×10^{-2})	3.8×10^{-2} (3.0×10^{-3})	1.69 (4.66)	2
3.50	0.30 (4.7×10^{-3})	2.1×10^{-2} (3.2×10^{-4})	2.23 (0.91)	2
3.90	0.17 (3.7×10^{-3})	1.2×10^{-2} (2.5×10^{-4})	3.92 (1.28)	2
4.30	0.06 (6.6×10^{-4})	4.5×10^{-3} (4.4×10^{-5})	*11.08 (1.99)	2
4.71	0.03 (6.6×10^{-4})	2.2×10^{-3} (4.5×10^{-5})	*17.25 (4.07)	2
5.13	0.01 (5.5×10^{-4})	8.5×10^{-4} (3.8×10^{-5})	*35.46 (8.88)	2

[a] The long lag times recorded for these pH values are most likely to be artefacts caused by the particularly low levels of detection throughout these series of results (values in parentheses represent the S.E.).

Table 2
Permeation data for salicylic acid across human skin from 14.48 mM solutions[a]

pH	Flux (μmol cm^{-2} h^{-1})	k_{obs} (cm h^{-1})	Lag time (min)	N
2.10	0.72 (5.7×10^{-2})	5.0×10^{-2} (3.9×10^{-2})	2.56 (4.92)	2
2.27	0.59 (1.6×10^{-2})	4.1×10^{-2} (1.1×10^{-3})	1.02 (1.70)	2
2.72	0.54 (9.2×10^{-3})	3.7×10^{-2} (6.3×10^{-4})	4.39 (1.07)	2
3.13	0.25 (6.2×10^{-3})	1.7×10^{-2} (4.3×10^{-4})	6.68 (1.56)	2
3.50	0.15 (5.0×10^{-3})	1.1×10^{-2} (3.5×10^{-4})	5.04 (2.02)	2
3.90	0.07 (1.4×10^{-3})	4.7×10^{-3} (9.9×10^{-5})	4.02 (1.29)	2
4.30	0.05 (1.0×10^{-3})	3.4×10^{-3} (7.1×10^{-7})	4.04 (0.93)	2
4.71	0.04 (1.7×10^{-5})	2.5×10^{-3} (1.1×10^{-6})	5.57 (3.74)	2
5.13	0.01 (1.2×10^{-5})	8.2×10^{-4} (8.6×10^{-7})	9.78 (5.92)	2

[a] Values in parentheses represent the S.E.

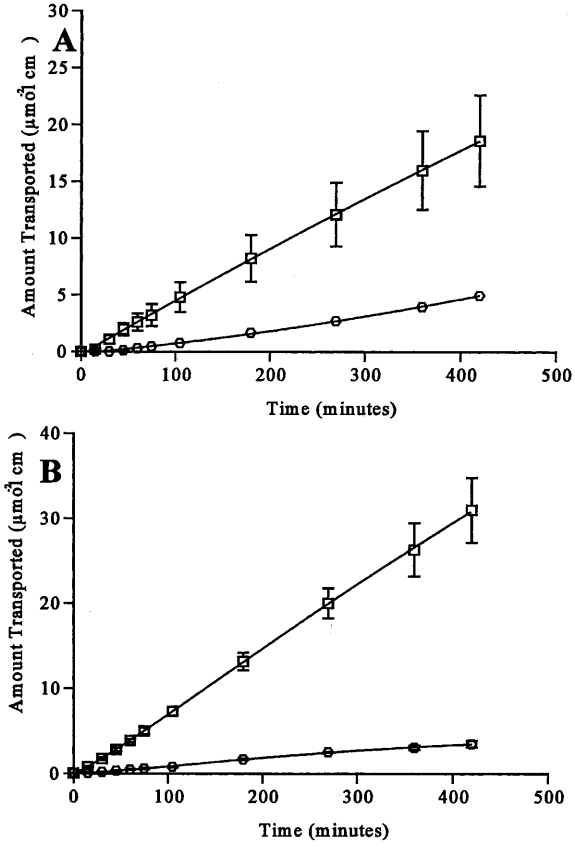

Fig. 1. The effect of pretreatment with 0.5 M lauric acid dimethylamide in isopropyl myristate upon the permeation of salicylic acid from saturated aqueous suspensions at pH 4.04 across human skin (○, Control; □, Pretreated skin; Error bars represent SEM). A McIlvaine buffer receiver pH 7.22; pH 4.04 donor phase, B McIlvaine buffer receiver pH 3.42; pH 4.04 donor phase.

against pH show a sigmoidal curve. There is a linear relationship between the fraction unionised $(1-\alpha)$ and flux. The total observed flux (J_{obs}) is dependent upon the flux of ionised (J_i) and unionised (J_u) species and the fraction of drug in each state $(1-\alpha,\ \alpha)$, thus $J_{obs} = \alpha J_i + (1-\alpha)\ J_u$, which may be linearised to $J_{obs}/\alpha = J_i + (1-\alpha)\ J_u/\alpha$. A plot of J_{obs}/α against $(1-\alpha)/\alpha$ enables the flux of the ionised and unionised species to be estimated from the intercept and slope, respectively (Irwin et al., 1990a). The low values for the intercept (J_i, $9.44\times10^{-3}\pm6.68\times10^{-3}$ μmol cm^{-2} h^{-1} for SR and $9.16\times10^{-3}\pm1.19\times10^{-2}$ μmol cm^{-2} h^{-1} for HS) is evidence that the ionised species has a negligible influence on flux. From the gradient of the line, the flux of the unionised species (J_u) is estimated as $1.19\pm3.01\times10^{-2}$ μmol cm^{-2} h^{-1} for SR and $0.76\pm2.90\times10^{-2}$ μmol cm^{-2} h^{-1} for HS. These values predict a flux of SA from 2 mg ml^{-1} donor solutions across SR at a pH where all the drug is totally unionised as 1.19 μmol cm^{-2} h^{-1} and 0.76 μmol cm^{-2} h^{-1} across HS. Experimental fluxes were in good agreement and were measured as 1.31 ± 0.065 μmol cm^{-2} h^{-1} at pH 0.44 across SR and 0.72 ± 0.057 μmol cm^{-2} h^{-1} at pH 2.1 across HS. The degree of ionisation and the degree of saturation of SA solutions differ as a consequence of pH. These results suggest that the change in flux is a direct consequence of pH, which controls the concentration of the undissociated species.

3.2. Permeation of salicylic acid from suspensions without a pH-gradient

The relationship between pH and the solubility of ionisable compounds can be derived from the Henderson-Hasselbalch equation. For a weak acid this provides ($S = S_o$ ($[H_3O^+] + K_a$)/$[H_3O^+]$), where S is the overall solubility of the compound and S_o is the solubility of the unionised species, the intrinsic solubility. For suspensions, this equation shows that the solubility of the unionised species, S_o is constant and independent of pH. The overall increase in solubility is related to the solubility of the ionised species and the degree of ionisation is related to the pH of the vehicle. The pH-partition theory predicts that the steady-state flux is dependent upon the concentration of the unionised molecule, therefore J_{obs} should remain constant throughout a pH range.

To find the true effect of pH, and the variable responsible for flux across absorption barriers, the concentration of undissociated molecules and the degree of saturation must remain constant over the pH range. This is achieved by using buffered saturated suspensions of SA over a pH range 0.08–4.71 as donors. It was found essential to check the pH of the suspension prior to use. This was especially so for high pHs where the solubility of SA is high. This precaution was necessary because, as SA dissolves, it modifies the pH of the buffer, and as the pH is adjusted, the SA either dissolves or precipitates, further changing the pH of the suspension. A suspension with a stable pH was prepared at least 24 h before use. Receiver solutions were McIlvaine buffers adjusted to a pH similar to that of the donor. The steady-state fluxes, permeability coefficients and lag times derived from suspension permeation profiles are summarised in Table 3 for SR and Table 4 for HS. Plots of the amount of SA permeated from aqueous suspensions against time and the steady-state flux against pH show that permeation is independent of pH. The mean steady-state flux was 1.94 ± 0.066 μmol cm^{-2} h^{-1} across SR over the pH range 0.08–4.71 and 1.10 ± 0.071 μmol cm^{-2} h^{-1} across HS over the pH range 1.84–4.71. The value for SR is somewhat higher than that forecast from the solution data. This may be due to reduced drive in the solution experiments, due to depletion of the donor phase. This underestimation could be expected as solution data for azidoprofen predicted a lower flux for saturated suspensions across SR (Naik, 1990). Ibuprofen solution data across hairless mouse skin also predicted a lower flux for saturated suspensions than that observed (Irwin et al., 1990a). The mean lag time for SR was 0.89 ± 0.25 min which emphasises the low resistance to diffusion of SR. The mean lag time with HS was 19.20 ± 3.2 min.

Table 3
Permeation data for salicylic acid across silastic rubber from saturated suspensions[a]

pH	Flux (μmol cm^{-2} h^{-1})	k_{obs} (cm h^{-1})	Lag time (min)	n
0.08	1.80 (0.064)	0.11 (3.9×10^{-3})	0.00 (2.04)	2
1.84	1.91 (0.027)	0.09 (1.2×10^{-3})	0.51 (0.81)	2
2.35	2.08 (0.044)	0.08 (1.7×10^{-3})	1.16 (1.23)	2
2.8	1.83 (0.053)	0.05 (1.4×10^{-4})	0.60 (1.67)	2
3.14	1.82 (0.034)	0.03 (6.4×10^{-4})	1.47 (1.09)	2
3.45	1.90 (0.023)	0.02 (2.2×10^{-4})	1.40 (0.71)	2
3.73	1.94 (0.048	0.01 (2.9×10^{-4})	0.00 (1.43)	2
4.17	1.94 (0.042)	0.003 (8.0×10^{-5})	0.56 (1.26)	2
4.71	2.38 (0.044)	0.001 (2.1×10^{-5})	2.39 (1.06)	2
Mean	*1.96	–	0.9	
Standard	*0.178	–	0.78	

[a] Values in parentheses represent the S.E. *Mean and S.D. of flux without pH 4.71 outlier value are 1.90 and 0.09, respectively.

Table 4
Permeation data for salicylic acid across human skin from saturated suspensions[a]

pH	Flux (μmol cm^{-2} h^{-1})	k_{obs} (cm h^{-1})	Lag time (min)	n
1.84	1.01 (0.019)	4.5×10^{-2} (8.4×10^{-4})	7.73 (4.34)	2
2.35	1.42 (0.024)	5.5×10^{-2} (9.4×10^{-4})	17.53 (3.95)	2
2.80	1.22 (0.013)	3.3×10^{-2} (3.5×10^{-4})	30.53 (2.46)	2
3.14	0.93 (0.017)	1.7×10^{-2} (3.2×10^{-4})	10.33 (4.26)	2
3.45	1.19 (0.015)	1.2×10^{-2} (1.5×10^{-4})	13.16 (2.95)	2
3.73	1.24 (0.021)	6.1×10^{-3} (1.3×10^{-4})	26.51 (3.89)	2
4.17	0.90 (0.011)	1.7×10^{-3} (2.0×10^{-5})	16.74 (2.80)	2
4.71	0.84 (0.022)	3.9×10^{-4} (1.0×10^{-5})	31.04 (6.03)	2
Mean	1.09	–	19.20	
S.D.	0.202	–	9.083	

[a] Values in parentheses represent the S.E.

The observed permeability coefficient (k_{obs}) is calculated by dividing J_{obs} by the overall solubility of SA which increases with pH due to the increasing concentration of the ionised species. As the ionised species does not contribute to J_{obs}, k_{obs} decreases with increasing pH following a sigmoidal relationship. If J_{obs} was divided by S_o the solubility of the permeating unionised species then, as is observed in practice, k_{obs} would remain constant throughout the pH range examined.

The results demonstrate that the flux of SA across HS and SR is proportional to the concentration of unionised molecules in the donor phase. The experimental protocol used eliminated the potential effects of a pH-gradient by the utilisation of donors and receivers of similar pH. Under these conditions, permeation followed the pH-partition theory, the transmembrane permeation was essentially due to the unionised species and the contribution of the ionised species was negligible. The results comparing flux through HS and SR show that the trends and profiles were similar. The rates of permeation through HS were lower than that observed through SR. For solution data, the average steady-state flux (J_{obs}) through HS was 70% of the rate through of SR and for suspensions, the steady-state flux through HS was 56% that of SR. This compares well with the previous data (Nacht and Yeung, 1985) where SA from saturated solutions was found to permeate through HS at rates 58% of those recorded through SR. Many permeation investigations are of a comparative nature, therefore the results obtained in this study would indicate that SR is a useful tool to investigate and optimise vehicle pHs of topical SA formulations.

3.3. Permeation of salicylic acid from suspensions using a pH-gradient

To study the ion-pair effects of putative enhancers, a pH-gradient is essential. A suitable pH-gradient to test the ion-pair hypothesis is a donor at approximately pH 4 and a receiver at approximately pH 7 (Hadgraft et al., 1985). A series of experiments were performed to investigate whether a pH-gradient could effect the SA flux across HS and SR from saturated suspensions. Initially a series of donor suspensions of various pH values were used, delivering into a receiver solution at pH 7.22. Secondly, a donor suspension at pH 4.04 delivering into receiver solutions over a range of pH values was studied. Plots of the amount of SA permeated from aqueous suspensions against time and the steady-state flux against pH for all these experiments showed that permeation is related to the concentration of unionised molecules and is independent

of pH or pH-gradients. The mean flux for SA from donor aqueous suspensions over a pH range of 0.29–4.91 across SR into a pH 7.22 receiver was 1.50 ± 0.025 μmol cm^{-2} h^{-1}. The mean flux for SA from similar donor suspensions, pH range 2.75–4.68 across HS into a pH 7.22 receiver was 0.92 ± 0.034 μmol cm^{-2} h^{-1}. The permeation of salicylic from a pH 4.04 suspension across SR into receivers formulated over a pH range 0.44–8.13 acid, has a mean flux of 1.44 ± 0.048 μmol cm^{-2} h^{-1}. The flux from similar pH 4.04 SA suspensions across HS into a pH 7.22 receiver was 0.65 ± 0.141 μmol cm^{-2} h^{-1} and into a pH 3.40 receiver was 0.64 ± 0.069 μmol cm^{-2} h^{-1}. The pH of the receiver did not affect the transport of SA from the donor and the membrane itself is most probably the rate-limiting step.

Results showed that using this experimental set-up, SA flux was not influenced by a pH-gradient with respect to either the donor or receiver pH. These results provided further evidence that transport of SA obeyed the pH-partition hypothesis. Permeation studies using HS and SR as model membranes to study the influence of ionisation gave similar profiles, demonstrating the suitability of the SR model for this type of study.

The results from this study demonstrate that the topical delivery of a model ionogenic permeant, SA, obeys the pH-partition theory and transdermal permeation is due to the unionised species. This is true over a pH range of 0.08–4.17 for SR and pH 1.84–4.71 for HS. The contribution to flux from the ionised species is insignificant and does not have a role in transdermal delivery. These results contrast with those of (Loftsson, 1985) who proposed that increased ionisation can promote topical delivery of SA. This was based upon the observation of increasing flux with higher donor pH. However, these contrasting conclusions may be because SA in its unionised form has a high partition coefficient into oily phases. The partition coefficient of the unionised form (P_u) into IPM is 28.26 (Irwin and Smith, 1991) and the partition coefficient of Sa into IPM at pH 3.96 is 2.20. This would imply that SA should partition effectively through the stratum corneum. Results from this current study show that SA can be detected permeating across HS after 15 min and a steady-state permeation is achieved after 45 min. The assessment of the permeation profile in Loftsson's work from 3 to 12 h seems an unnecessarily long time to leave the SA in contact with the skin. Especially as hairless mouse skin has been shown to be more permeable than HS with acetyl salicylic acid (Bronaugh et al., 1982). During these long periods, the absorption profiles could have been influenced by SA's keratolytic effect upon the stratum corneum. It is also difficult to assess the effect of pH upon flux with data from only 3 pH values, even if each pH experiment was replicated four times. It may have been appropriate to observe whether this profile continued at even higher pHs, for example pH 6 and 7. The results of this study show is that the ionic species does not contribute to transdermal flux, which is almost exclusively due to the unionised species.

3.4. Absorption enhancer potentiation of salicylic acid permeation from suspensions using a pH-gradient

A pH-gradient is essential for an ion-pair facilitated transport mechanism (Hadgraft et al., 1985). If the effect of an absorption enhancer is greater in the presence of a transmembrane pH-gradient than it is without the pH-gradient, then the mode of action may be, to some extent, due to ion-pairing. If the enhancer effect is similar with and without the transmembrane pH-gradient, then the mode of action is more likely to be due to partition effects, with little or no influence from ion-pairing. A comparison of absorption enhancer activity with and without a transmembrane pH-gradient would help expose the likely mechanism of this model.

In order to determine the likely mechanism of absorption enhancer action, saturated suspensions of SA buffered to pH 4.04 were used as donors. The permeation of SA from this donor across SR and HS was evaluated following enhancer pretreatment. Receiver solutions were buffered to either pH 7.22 to provide a transmembrane pH-gradient to facilitate ion-pair formation, or pH 3.40 to ensure that ion-pair formation could not occur. The flux, observed premeation coefficient

Table 5
Permeation data following enhancer pretreatment of salicylic acid from saturated suspensions at pH 4.04 across silastic membrane into a buffer receiver at ph 7.22[a]

Pretreatment[b]	Flux (μmol cm^{-2} h^{-1})	k_{obs} (cm $h^{-1} \times 10^3$)	Flux ratio[c]	n
None	1.49 (0.047)	6.62 (0.208	1.00 (0.029)	6
PG	1.52 (0.035)	6.79 (0.154)	1.02 (0.023)	3
IPM	17.27 (0.836)	76.90 (3.720)	11.61 (0.562)	3
CDA in PG	1.50 (0.067	6.68 (0.297)	1.01 (0.045)	3
CDA in IPM	16.65 (0.880	74.14 (3.916)	11.19 (0.591)	3
LDA in PG	3.50 (0.190)	15.59 (0.844)	2.35 (0.127)	3
LDA in IPM	15.54 (1.714)	69.17 (7.632)	10.44 (1.152)	3
DCA in PG	1.69 (0.039)	7.54 (0.174	1.14 (0.026)	3
DCA in IPM	13.75 (0.321)	61.23 (1.429)	9.24 (0.216)	3

[a] Values in parentheses are the standard errors of the mean; CDA, caproic acid *N*,*N*-dimethylamide; LDA, lauric acid *N*,*N*-dimethylamide; DCA, dodecylamine.
[b] Enhancer concentrations are 0.5 M in their respective vehicles.
[c] Flux ratio is the flux following pretreatment with enhancer/no pretreatment.

and flux ratio for SA permeation from a pH 4.04 saturated donor following enhancer pretreatments across SR with a transmembrane pH-gradient are summarised in Table 5 and without a transmembrane pH-gradient in Table 6. The efficacy of penetration enhancers was quantified by dividing SA flux after enhancer pretreatment by flux with no pretreatment. This is defined as the flux ratio (otherwise termed the enhancement factor, (Bonina and Montenegro, 1992; Ruland and Kreuter, 1992).

The flux ratios for SA across SR using enhancers in IPM are approximately ten times greater than those in PG vehicles. After pretreatment with IPM, SR became appreciably distorted, implying that the additional potentiation attributed to IPM over PG is due to membrane damage. This effect shows that SR is an inappropriate model for determining the effect of enhancers in IPM. Pretreatment with PG alone did not have a significant enhancing effect upon SR. 0.5 M CDA in PG had very little effect upon the

Table 6
Permeation data following enhancer pretreatment of salicylic acid from saturated suspensions at pH 4.04 across silastic membrane into a buffer receiver at pH 3.40[a]

Pretreatment[b]	Flux (μmol cm^{-2} h^{-1})	k_{obs} (cm $h^{-1} \times 10^3$)	Flux ratio[c]	n
None	1.23 (0.065)	5.46 (0.288)	1.00 (0.061)	3
PG	1.39 (0.045)	6.19 (0.199)	1.13 (0.036)	3
IPM	14.45 (0.204)	64.35 (0.908)	11.79 (0.166)	3
CDA in PG	1.46 (0.011)	6.49 (0.047)	1.19 (0.009)	3
CDA in IPM	14.28 (1.172)	63.58 (5.217)	11.65 (0.956)	3
LDA in PG	3.62 (0.296)	16.11 (1.318)	2.95 (0.241)	3
LDA in IPM	11.72 (1.041)	52.18 (4.637)	9.56 (0.849)	3
DCA in PG	1.11 (0.038)	4.94 (0.167)	0.90 (0.031)	3
DCA in IPM	8.88 (0.648)	39.54 (2.884)	7.24 (0.528)	3

[a] Values in parentheses are the S.E. of the mean; CDA, caproic acid *N*,*N*-dimethylamide; LDA, lauric acid *N*,*N*-dimethylamide; DCA, dodecylamine.
[b] Enhancer concentrations are 0.5 M in their respective vehicles.
[c] Flux ratio is the flux following pretreatment with enhancer/no pretreatment.

penetration of SA through SR into receivers of either pH. The flux ratio of DCA in PG into the pH 3.40 receiver was 0.90 ± 0.031, this increases to 1.14 ± 0.026 in the pH 7.22 experiments where a pH-gradient is employed. SR does not appear to be damaged by DCA, unlike biological membranes where detrimental effects have been reported (Naik et al., 1993). The flux into the pH 7.22 receiver is an 11% increase in flux over the PG vehicle. This increase in flux over the pH 3.40 experiments is significant ($P < 0.001$). The increase in flux observed by both CDA and LDA into receivers at the two pHs was not significantly different ($P > 0.5$). Across SR, the enhancement due to DCA is facilitated by a pH-gradient, whereas that by LDA and CDA is not. This would imply that the effect of DCA is due, at least to some extent, to ion-pairing, whereas the enhancement due to CDA and LDA is due to partitioning phenomena alone. The medium chain LDA (C_{12}) in PG enhanced permeation of SA by a flux ratio of 2.35 ± 0.127 into the pH 7.22 receiver and 2.95 ± 0.241 into the pH 3.40 receiver. In comparison, the shorter chain CDA (C_6) in PG which enhanced SA by a flux ratio of 1.01 ± 0.045 into the pH 7.22 receiver and 1.19 ± 0.009 into the pH 3.40 receiver. The difference in the enhancing effect between LDA and CDA is significant ($P = 0.0046$, pH 7.2 receiver, $P = 0.0181$, pH 3.4 receiver). This agrees with data for the enhancement of naproxen across rat skin by a series of *N*,*N*-dimethylamides where the C_8–C_{14} range were found to give the maximum enhancement (Irwin et al., 1990b). These results follow a similar trend to SA partition data (Irwin and Smith, 1991).

A similar series of experiments was undertaken using HS. The flux, observed permeability coefficient and flux ratio for SA permeation from a pH 4.04 saturated donor following enhancer pretreatment across HR with a transmembrane pH-gradient are summarised in Table 7 and without a pH-gradient in Table 8. PG has a limited effect upon SA permeation across HS. This is similar to that observed with SR. However, in direct contrast to the SR results, IPM had a negligible effect upon HS. IPM does not disrupt the integrity of HS as it does SR. This is confirmed from the macroscopic detail of the skin, where no obvious destruction was observed. This is anticipated, as IPM is frequently used in cosmetic and pharmaceutical topical formulations as a vehicle and moisturising agent (Croda Chemicals Ltd., 1995, 1996). The LDA in PG had a flux ratio of 3.53 ± 0.718 utilising a pH-gradient and 2.93 ± 0.255 into the pH 3.40 receiver (Fig. 1). These results did not represent a significant difference ($P = 0.3568$, Table 9). Bar chart comparisons of the enhancer flux ratios into the two buffers are shown in Fig. 2. It can be concluded that the

Table 7
Permeation data following enhancer pretreatment of salicylic and from saturated suspensions at pH 4.04 across human skin into a buffer receiver at pH 7.22[a]

Pretreatment[b]	Flux (μmol cm^{-2} h^{-1})	k_{obs} (cm $h^{-1} \times 10^3$)	Flux ratio[c]	*n*
None	0.65 (0.163)	2.89 (0.725)	1.00 (0.251)	4
PG	0.79 (0.014)	3.51 (0.062)	1.21 (0.021)	4
IPM	0.87 (0.002)	3.86 (0.011)	1.33 (0.004)	2
LDA in PG	2.29 (0.466)	10.19 (2.075)	3.53 (0.718)	4
LDA in IPM	3.56 (0.387)	15.87 (1.723)	5.50 (0.597)	4
DCA in IPM	37.52 (1.502)	167.05 (6.689)	57.84 (2.316)	4
Azone in PG	1.64 (0.155)	7.28 (0.692)	2.52 (0.293)	3
OA in PG	2.07 (0.189)	9.22 (0.842)	3.19 (0.357)	3
TC in PG	0.76 (0.052)	3.39 (0.229)	1.17 (0.097)	3

[a] Values in parentheses are the S.E. of the mean; Rates are normalised to none pretreated controls).
[b] Enhancer concentrations are 0.5 M in their respective vehicles.
[c] Flux ratio is the flux following pretreatment with enhancer/no pretreatment.

Table 8
Permeation data following enhancer pretreatment of salicylic acid from saturated suspensions at pH 4.04 across human skin into a buffer receiver at pH 3.40[a]

Pretreatment[b]	Flux (μmol cm^{-2} h^{-1})	k_{obs} (cm $h^{-1} \times 10^3$)	Flux ratio[c]	n
None	0.64 (0.080)	2.85 (0.356)	1.00 (0.125)	4
PG	0.65 (0.033)	2.89 (0.148)	1.02 (0.052)	3
LDA in PG	1.87 (0.163)	8.35 (0.725)	2.93 (0.255)	3
LDA in PG	5.53 (0.667)	24.62 (2.969)	8.65 (1.043)	4
DCA in IPM	18.45 (0.765)	82.12 (3.405)	28.85 (1.196)	3
Azone in PG	1.06 (0.021)	4.70 (0.091)	1.65 (0.032)	3
OA in PG	1.94 (0.169)	8.64 (0.754)	3.04 (0.265)	3
TC in PG	0.80 (0.119)	3.57 (0.530)	1.26 (0.186)	3

[a] Values in parentheses are the S.E. of the mean (rates are normalised to none pretreated controls).
[b] Enhancer concentrations are 0.5 M in their respective vehicles.
[c] Flux ratio is the flux following pretreatment with enhancer/no pretreatment.

enhancement observed is not due to ion-pair facilitation. These results are in agreement with the physicochemical data for *N*,*N*-dimethylamides, which are very weak bases with pK_a values of less than 1 (Higuchi et al., 1962; Adelman, 1964). Under these experimental conditions, they would not be expected to be sufficiently ionised to promote ion-pairing. Enhancement by *N*,*N*-dimethylamides is probably due to a modification of drug partition into the stratum corneum. DCA has a massive absorption-enhancing effect on SA through HS, with flux ratios of 57.84 ± 2.316 into the pH 7.2 receiver and 28.85 ± 1.196 into the pH 3.4 receiver. This degree of potentiation would be consistent with DCA causing a significant reduction in barrier resistance or destruction of the membrane (Naik et al., 1993). The amount transported verses time profile for DCA showed a marked reduction in flux after 3 h. This could be interpreted as a reversible process due to exhaustion of the enhancer. Another possible explanation is a thermodynamic effect due to the reduction of the concentration gradient of the SA. The total quantity of SA, (approximately 360 μmol, 4.7 mg) transported into the pH 7.2 receiver gave a final concentration in the receiver cell of 6.2% of the donor cell concentration. It is also possible that with this amount of SA transported, the donor solution was no longer saturated. This would have reduced the true 'sink' conditions of the experiment. Without a pH-gradient, Azone demonstrated a flux ratio of 1.65 ± 0.032, implying that this aspect of Azone enhancement is achieved *via* a direct membrane effect. Azone is a non-polar molecule that partitions directly into the lipid regions of the stratum corneum and disrupts the lipid domains. The increased fluidity reduces the diffusional resistance to drugs (Barry, 1991; Beastall et al., 1988). Azone has also been associated with the ion-pairing theory of facili-

Table 9
The probability of a difference between the level of enhancement of salicylic acid permeation across human skin by absorption enhancers with and without a pH-gradient[a]

Absorption enhancer	None	PG	LDA in PG	LDA in IPM	DCA in IPM	Azone in PG	OA in PG	TC in PG
Probability P $(T \leq t)$ two-tail	0.9612	0.0395	0.3568	0.0532	0.0001	0.0915	0.6778	0.7797

[a] Two tailed heteroscedastic *t*-test; CDA, caproic acid *N*,*N*-dimethylamide; LDA, lauric and *N*,*N*-dimethylamide; DCA, dodecylamine; OA, oleic acid; TC, transcutol).

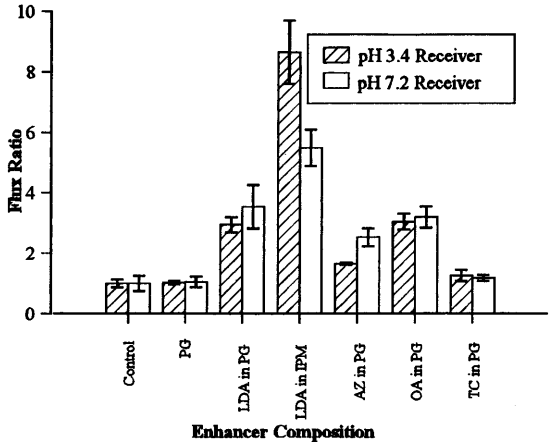

Fig. 2. The effect of enhancer pretreatment on the flux ratio of salicylic acid from saturated suspensions at pH 4.04 across human skin with and without a pH-gradient (error bars represent the S.E. of the mean).

tated drug transport (Hadgraft et al., 1985). The results utilising a pH-gradient showed an enhancement of transdermal flux with a flux ratio of 2.52 ± 0.293. Using Azone, although the flux enhancement with a pH-gradient was slightly greater than the flux enhancement without a pH-gradient, the difference was not significant ($P = 0.0915$, Table 9). This suggests that Azone does not enhance transdermal flux of SA via an ion-pairing mechanism. In the PG vehicle, OA is the most efficient penetration enhancer. The flux ratio was similar with and without a pH-gradient. These results support the hypothesis that, in some cases, the action of Azone can be explained by partition effect alone (Irwin and Smith, 1991). The OA results are consistent with the hypothesis that OA exerts its action by the disruption of the lipid layer. Once OA has penetrated into the stratum corneum lipids its 'kinked' structure due to the *cis*-double bond disrupts and increases the fluidity of the lipid packing. Co-solvents such as PG have a synergistic action with OA. This may be due to the co-solvent's ability to reduce the polarity of the aqueous regions of the stratum corneum, so increasing the ability of the stratum corneum to solubilise OA. TC in PG had a very limited apparent effect upon the flux of SA across HS. However, it has been shown that TC increases the flux of model drugs into the human stratum corneum possibly by increasing the solubility of the penetrant in the stratum corneum (Harrison et al., 1996), but the drugs remain in this layer as a depot (Panchagnula and Ritschel, 1991). TC is believed to cause intercellular lipid swelling without altering the multiple bilayer structure. This effect results in the drug depot developing within the stratum corneum, hence the increased penetration of drugs into the skin and the decreased permeation across the skin. The experimental method used in this study only measured the drug penetrating through the skin so this depot effect of TC would not have been observed. This mode of action may be usefully exploited as a co-solvent of other enhancers such as Azone or OA.

Investigations using HS confirmed that OA and Azone are valuable compounds for enhancing transdermal penetration. Both demonstrate actions of lipid perturbation, but comparing enhancement potential with and without a pH-gradient, neither compound demonstrated potential for enhanced permeation due to ion-pairing. LDA also showed potential for enhancing transdermal penetration. An increased partition into the absorption barrier is a likely mode of action. The system did not show evidence of an ion-pair facilitated transport. The increase in transdermal flux of SA with LDA as an enhancer was further potentiated using IPM as a vehicle as opposed to PG. TC in PG and the two vehicles alone, IPM and PG had minimal effects upon the rate of flux. The evidence from this series of experiments with and without pH-gradients demonstrate that, under certain conditions, the transdermal penetration enhancement of a number of topical enhancing compounds, including Azone and OA, can be explained without recourse to ion-pair phenomena.

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