In Vitro Percutaneous Absorption of Arildone, a Highly Lipophilic Drug, and the Apparent No-Effect of the Penetration Enhancer Azone in Excised Human Skin

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Purpose. Arildone, a novel lipophilic antiviral drug when evaluated in Clinical Trials showed limited skin absorption and antiviral efficacy. These studies were conducted to explain the apparent poor absorption characteristics and attempt to promote skin absorption by using Azone, a penetration enhancer. ***Methods.*** Standard *in vitro* skin permeation methods using excised human skin were employed to characterise the absorption of Arildone. ^{14}C-Arildone was used to estimate the distribution in skin layers by scintigraphic and autoradiographic procedures. ***Results.*** The aqueous solubility and distribution constant values for Arildone were 2 $\mu g\ ml^{-1}$ and 5×10^5 (isopropyl myristate/water), respectively. Absorption through full thickness skin or stratum corneum-viable epidermal membranes (diffusional resistant dermis removed), from a propylene glycol vehicle, was slow and the addition of Azone had no effect on the permeation rate. Distribution studies showed accumulation of Arildone in the stratum corneum. The concentration of Arildone in the viable epidermis was estimated from sectioning the skin and was found to be in sufficient amounts (400 $\mu g\ cm^{-3}$) to have potential antiviral activity. ***Conclusions.*** The apparent accumulation of Arildone in the stratum corneum suggested that the hydrophilic skin region presented the main barrier to permeation. Azone which affected the permeability of the stratum corneum was therefore not effective at enhancing Arildone absorption. Vehicles which readily permeate and enhance the transfer of lipophilic drugs from the stratum corneum into the viable epidermis were recommended.

KEY WORDS: arildone; antiviral; percutaneous absorption; Azone; penetration enhancer; autoradiography.

INTRODUCTION

Many drug candidates have been investigated for their potential to permeate the skin barrier but with varying success. Few compounds of therapeutic interest possess the optimal physicochemical properties to penetrate readily through the stratum corneum and into the viable epidermis and therefore various methods have been employed to promote skin absorption of poorly penetrating drugs (1). One method is to disrupt, temporarily, the barrier properties of the stratum corneum by including a penetration enhancer in a formulation (2).

Azone® (1-dodecylazacycloheptan-2-one) is an agent that enhances the absorption of various compounds across the skin (3,4,5,6). The enhancing effect is thought to be a result of disruption in the lipid domain of the stratum corneum (7).

This report describes the skin permeation of a novel lipophilic antiviral compound Arildone 4[-(2-chloro-4-methoxy)phenoxy] hexyl-3,5-heptanedione and the influence of Azone on its permeation behaviour.

Arildone is active against para-influenza, respiratory syncytial and herpes simplex viruses (8). The antiherpetic activity was of particular interest as there are few commercial antiviral drugs available. The primary indication for Arildone was for topical therapy of herpetic skin lesions.

In vivo animal studies and clinical trials examining the fate of Arildone following cutaneous application, have produced conflicting results on the extent of Arildone absorption. Topical application of radiolabelled Arildone to mice resulted in 94% of the dose being excreted in 3 days (9). By contrast, tolerance and availability studies with Arildone in man administered as a cream and a solution in dimethylsulphoxide, demonstrated low and sporadic absorption in which dimethylsulphoxide, a well characterised penetration enhancer (1) had minimal effect (10).

The poor absorption in humans may have been due to formulation effects or to the physicochemical properties of Arildone or both. Differences between the skin permeability between mice and humans would also explain the divergent absorption data reported in these two species. The disposition of Arildone in humans has not been characterised, although it is extensively metabolised in various animal species (9). The apparent variable absorption may also be partly due to metabolism of Arildone to barely detectable levels. A double blind placebo controlled trial of Arildone cream failed to show efficacy in patients with recurrent herpes infections (11). The reason for the lack of efficacy was not known although it was postulated that due to the dosing schedule, insufficient drug levels were achieved during the critical first stages of a lesion appearing. Alternatively, the cream formulation may not have provided adequate drug delivery.

The objective of the work described in this report was to explain the apparent poor absorption of Arildone observed in human studies (10,11) and to recommend an alternative formulation approach. To this end, the permeation of Arildone was evaluated using *in vitro* human skin permeation and autoradiographic techniques. Before conducting skin permeation studies, the solubility and partitioning behaviour of Arildone was determined.

MATERIALS AND METHODS

Full experimental details of the following procedures have been described previously (12). Arildone and ^{14}C-Arildone (radiolabel purity of 95.7%, and a specific activity of 0.41 $MBq\ mg^{-1}$) were obtained from Sanofi Research Division, Alnwick, UK. Azone was a gift from Whitby Research Inc. All other materials were obtained from local suppliers and were used as received.

The solubility of Arildone was determined in propylene glycol (PG)-water mixtures saturated with excess Arildone.

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The distribution constant (K_D) between isopropyl myristate (IPM) and PG-water mixtures was determined using a stirred flask technique. Drug vehicle combinations were applied to the skin surface as a film in small volume ethanolic solutions (finite dose technique). Upon evaporation of the ethanolic component each vehicle was saturated with Arildone thereby providing optimal thermodynamic activity.

Female and non-pileous male midline abdominal skin was obtained from cadavers at autopsy whose ages ranged from 60 to 90 years and was used immediately or was frozen until required. For the permeation experiments involving isolated stratum corneum epidermal (sc-ep) and dermal membranes, the skin was heat treated to facilitate removal of the epidermal tissue from the dermis, as described by Kligman and Christophers (13).

Skin membranes were mounted on standard Franz type diffusion cells with an available skin surface area of 0.44 cm^2. The receptor fluid maintained at 37°C, was 50% v/v ethanol in water. Ethanol was added to provide sink conditions. The investigation of metronidazole permeation used aqueous isotonic phosphate buffer, pH 7.4, as receptor phase containing 0.001% w/v phenyl mercuric nitrate. Samples of receptor phase were analysed directly using HPLC. The radiolabelled study used cadaver skin and the same permeation apparatus as above.

Treatment of Permeation Data

The cumulative amount of Arildone reaching the receptor phase per unit per area of skin was plotted against time. The rate of permeation of Arildone through the skin was such that over the time course of most of the experiments a pseudo-steady state flux was achieved. This allowed the mean pseudo-steady state flux, J, to be calculated from the linear portion of the slope using linear regression analysis.

RESULTS AND DISCUSSION

The lipophilic characteristics of Arildone are illustrated in Figure 1 which shows the relationship between distribution constant, K_D, and solubility as a function of PG-water fraction. Extrapolation to zero PG fraction enabled estimates of K_D between IPM and water, and the aqueous solubility to be determined. The relative hydrophilic and lipophilic characteristics of IPM are reported to mimic skin lipids (14). These values were 5×10^5 ($\log K_D = 5.7$) and 2 $\mu g\ ml^{-1}$ respectively. The ability of a molecule to permeate through the lipophilic and aqueous domains of the skin will depend upon its partitioning behaviour (15) and based up a $\log K_D$ of 5.7, Arildone would not be expected to be readily absorbed.

The permeation of Arildone from PG and 5% Azone/PG vehicles through full thickness skin is illustrated in Fig. 2. It is apparent from the permeation profiles that Azone did not enhance the permeation of Arildone. This lack of enhancement is illustrated further by permeation data in Table I, where there was no apparent increase in the mean pseudo-steady state flux (J) in the presence of Azone with or without PG. The apparent linearity of these profiles indicated that permeation was slow. Other workers have observed a synergistic enhancing effect when Azone is combined with PG (5,6). No such synergism was observed in these experiments. Evaporation of the ethanolic component of the vehicles resulted in Arildone exceeding the solubility limit in the PG vehicle and this may in part have reduced the absorption rate. Substitution of PG with dimethlyformamide (DMF) in which Arildone was present at approximately 12% of its solubility limit, did not alter the absorption rate compared to PG, and again no enhancing effect was observed with Azone (Table I).

The dermis may present a substantial barrier for hydrophobic drugs (16), Arildone being such an example. Favourable *in vivo-in vitro* correlation for absorption of hydrophobic drugs has been achieved by removing most of the dermis from excised skin *in vitro* (17). Either separated epidermis or skin sliced to within 200 μm of the viable epidermis thereby leaving the papillary layer intact has been used (18,19).

It was possible that the diffusional resistance offered by the dermis was masking any enhancement produced by

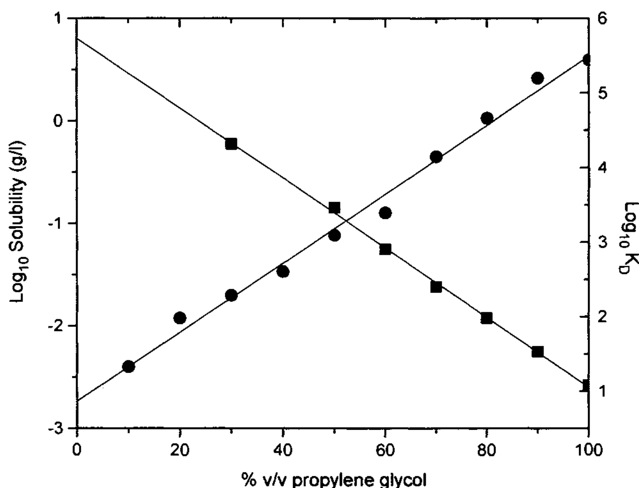

Fig. 1. Plot of solubility (●) and distribution constant (IPM/PG-water mixtures, K_D, ■) for Arildone against %v/v propylene glycol in water at 25°C.

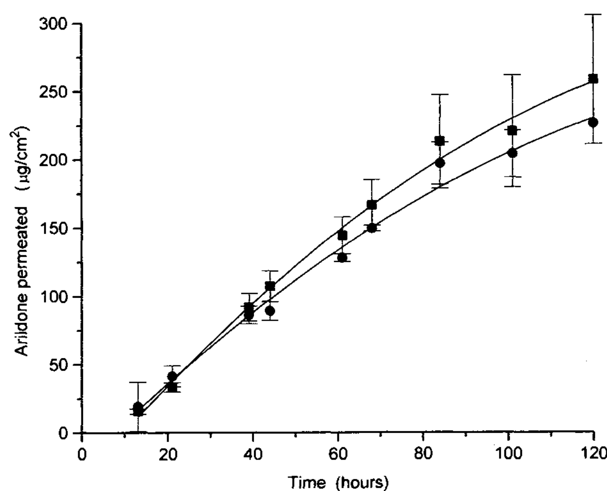

Fig. 2. Permeation of Arildone through full thickness skin (deposited as an ethanolic film) from 5% Azone/PG[a] (●) and PG[b] (■). a = 100 μl ethanol containing 2 mg Arildone, 0.5 μl Azone, 10 mg PG. b = 100 μl ethanol containing 2 mg Arildone, 10 mg PG. Each data point is the mean (n = 3) with standard deviation.

Table I. Mean Pseudo-Steady State Flux, J, $\mu g\ cm^{-2}\ hr^{-1}$, (± Standard Deviation) for Arildone Permeation from Azone/PG and DMF Vehicles Through Full Thickness Skin, n = Number of Experiments

Experiment	Vehicle	J	n
1	Arildone only	1.1 (0.4)	3
	Azone	2.7 (0.6)	3
2	5% Azone/PG	2.6 (0.7)	3
	PG	2.5	2
3	PG	2.4	2
	17% Azone/PG	2.2	2
	33% Azone/PG	1.0	2
4	PG	2.6 (0.7)	3
	2% Azone/PG	2.5 (0.5)	3
5	DMF	2.2 (0.1)	3
	5% Azone/DMF	1.7 (0.3)	3

Azone. The permeation of Arildone through isolated sc-ep was investigated to confirm whether the dermis was contributing to the diffusional barrier. To validate the technique, the permeation of metronidazole a relatively polar drug was examined. The permeation profiles through sc-ep membrane, isolated dermis and full thickness skin are given in Fig. 3. Permeation through dermis was rapid as expected with minimal diffusional resistance, while the permeation through sc-ep and full thickness skin was slower, controlled by the stratum corneum. The heat separation method used to prepare the isolated sc-ep membranes appeared not to affect the integrity of these membranes.

The experiment was repeated with Arildone (Fig. 4). The permeation through sc-ep was relatively rapid with equilibrium occurring at 100 hours, although only about 37% of the applied dose reached the receptor phase. The permeation through the full thickness skin was slower, while that through the isolated dermis was slowest of all. These findings confirmed that the dermal region provided the greatest resistance to diffusion of Arildone under *in vitro* conditions.

Azone was investigated further using sc-ep membranes in place of full thickness skin (Fig. 5). Figure 5 shows that no enhancing effect is demonstrated when the permeation profiles from 5% Azone/PG and PG vehicles are compared. An approximate two-fold difference in the permeation rates for Arildone in PG through sc-ep membranes (Fig. 3 and 4) was observed. This was attributed to the use of skin from different donors.

The poor skin absorption of Arildone is likely to be due to unfavourable partitioning into the more hydrophilic regions of the viable epidermis and upper dermis (papillary layer). In addition, possible accumulation of Arildone may be expected to occur in the stratum concern to form a depot. Possible accumulation of Arildone in the stratum corneum was determined by quantifying ^{14}C-Arildone in lateral skin sections and visually by autoradiography. Table II summarises the activity in the stratum corneum, viable epider-

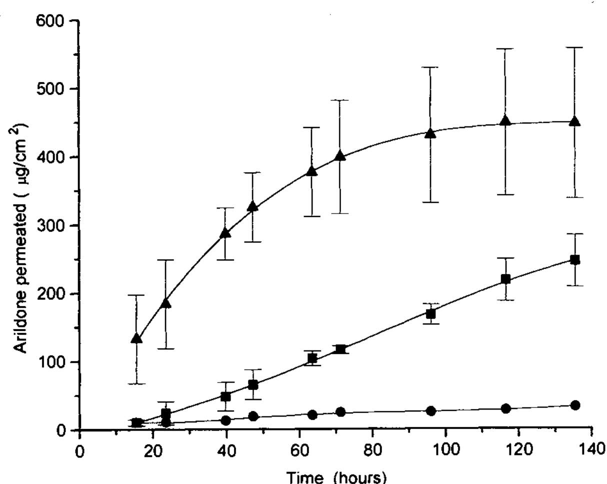

Fig. 4. Permeation of Arildone through separated skin layers from a PG vehicle deposited as an ethanolic film[a] onto full thickness skin (■), sc-ep (▲) and dermis (●). a = 25 µl ethanol containing 500 µg Arildone, 10 mg PG. Each data point was the mean of three experiments with standard deviation, except (●) where n = 2.

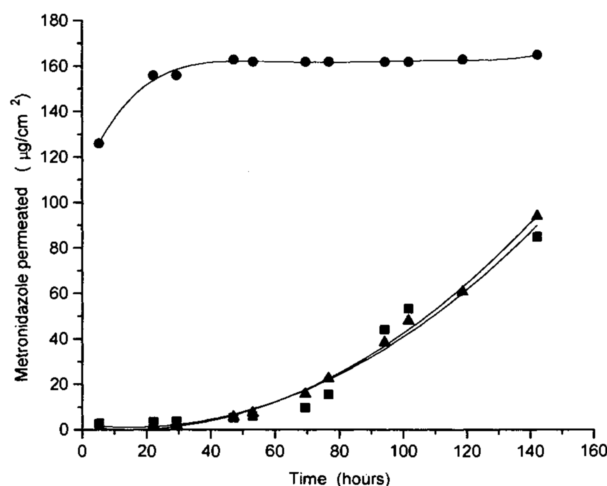

Fig. 3. Permeation of metronidazole through separated skin layers from a PG vehicle deposited as an ethanolic film[a] onto full thickness skin (■), sc-ep (▲) and dermis (●). a = 25 µl ethanol containing 75 µg metronidazole, 10 mg of PG. Each data point was the mean of three experiments.

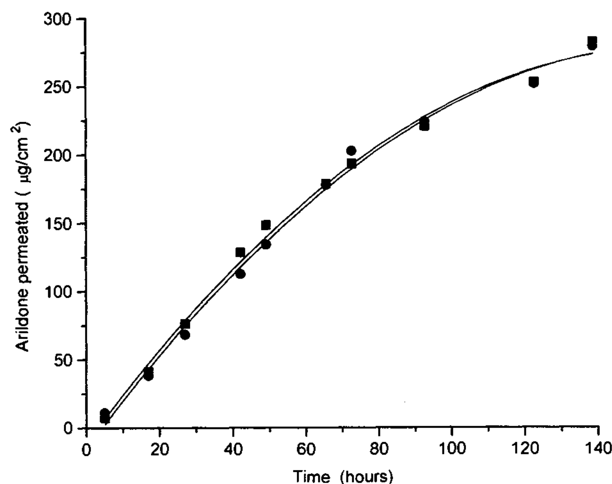

Fig. 5. Permeation of Arildone (deposited as an ethanolic film) through sc-epidermis from 5% Azone/PG[a] (●) and PG[b] (■) vehicles. a = 25 µl ethanol containing 500 µg Arildone, 0.5 µl Azone, 10 mg PG. b = 25 µl ethanol containing 500 µg Arildone, 10 mg PG. Each data point was the mean of three experiments.

Table II. Distribution of ^{14}C-Arildone Activity in the Skin Layers as a Percentage of the Total Skin Activity (Thickness of Each Layer is Shown in μm)

Time (hours)	Stratum corneum (10 μm)	Viable epidermis (20 μm)	Dermis (1400 μm)
6.5	95.5	4.2	0.3
95.5	92.3	6.7	1.0

mis and dermis. The relative thicknesses of stratum corneum and epidermal regions were estimated from microscopical examination and established literature values. The values in Table II represent a steep concentration gradient which was maintained even after about 100 hours exposure indicating that Arildone was retained in or on the stratum corneum. Visualisation of the activity as indicated by the density of black silver deposits reveals that the activity is associated with the stratum corneum (uppermost skin layer in Fig. 6). Surface washings prior to sectioning the skin collected 87% and 69% of the applied dose after 6.5 and 95.5 hours permeation time, respectively, indicating most of the applied dose had not permeated into the skin. The permeation rate was also monitored and a pseudo-steady state profile (linear) was obtained with J equal to 1.8 μg cm^{-1} hr^{-1} which compared favourably with data in Table I.

The level of activity illustrated in Figure 6, after accounting for the area of application and the thickness of skin sections in the viable epidermal/upper dermal region (30 μm skin depth below the stratum corneum) corresponded to a drug concentration of about 400 μg cm^{-3} of tissue after 6.5 hours. The minimum inhibitory concentration of Arildone for herpes simplex type 1 and type 2 viruses determined by tissue culture methods is reported to be 6 μg ml^{-1} (8). Therefore, the levels of drug attained in the permeation procedure were substantially greater than the minimum required for inhibiting viral replication. Although permeation through excised skin was slow and apparently unaffected by penetration enhancers the level of Arildone which could be attained within the viable epidermal region was sufficient, in theory, to have an inhibitory effect on viral replication *in vitro*. It was possible that therapeutic drug levels may have been achieved within the skin during the clinical trials described above (10) in which minimal systemic absorption was reported. However clinical studies using a cream failed to demonstrate efficacy (11). Insufficient drug release from the cream may have been the cause although the action of Arildone, to interfere early in the virus/host infection cycle may have meant that treatment was not initiated soon enough and for a sufficient period to be effective.

These studies demonstrate that the stratum corneum may provide a depot for drugs which are hydrophobic and lipophilic like Arildone. The co-administration of a solvent which increases the solubility of a hydrophobic drug in the aqueous region may aid absorption. PG appeared to do this in which potentially therapeutic quantities of Arildone were achieved in the viable epidermal-upper dermal region of the skin, a possible site of action for this drug. If similar hydro-

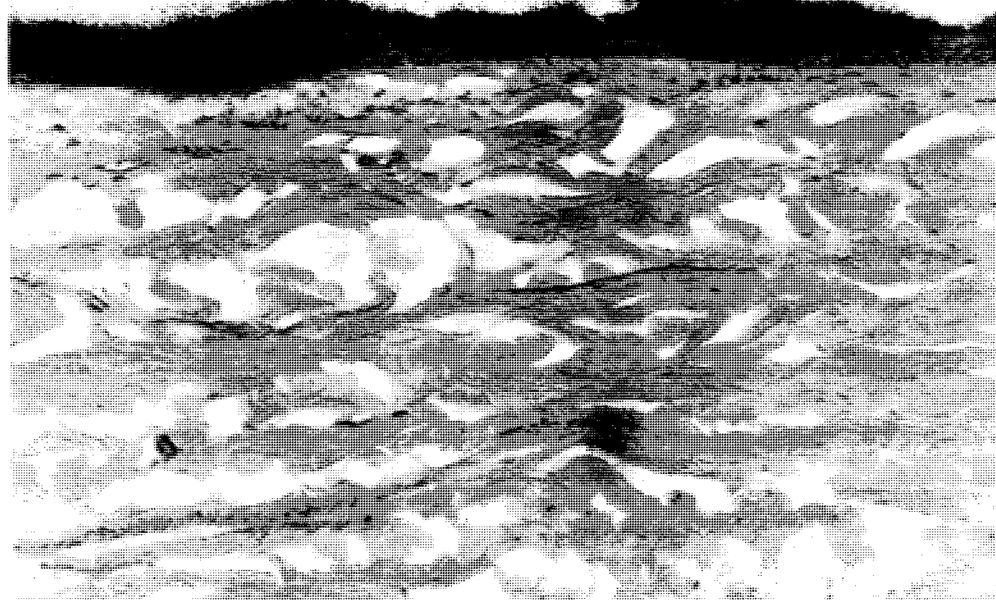

Fig. 6. Autoradiograph of a transverse skin section showing the distribution of ^{14}C-Arildone after 95.5 hours permeation time (×100 magnification). The activity is associated mainly with the stratum corneum. ^{14}C-Arildone was applied as a 35 μl solution in ethanol (480 μg, 0.2 MBq) following application of 10 mg PG in 20 μl ethanolic solution. Reduced to 65% for reproduction.

phobic drugs to Arildone are also considered for systemic delivery via the transdermal route, then penetration enhancers which promote transfer through aqueous regions would be more appropriate than compounds like Azone which perturb the stratum corneum.

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REFERENCES

1. B.W. Barry. *Dermatological Formulations: Percutaneous Absorption. Drugs and the Pharmaceutical Sciences,* Volume 18, J. Swarbrick (Ed.) Marcel Dekker, New York, 1983.
2. J. Hadgraft. Penetration enhancers for percutaneous absorption. *Pharm. Int.* 5:252-254 (1984).
3. R.B. Stoughton. Enhanced percutaneous penetration with 1-dodecylazacycloheptan-2-one. *Arch. Dermatol.* 118:474-477 (1982).
4. S.L. Spruance, M. McKeough, K. Sugibayashi, F. Robertson, P. Gaede and D.S. Clark. Effect of Azone and propylene glycol on penetration of trifluorothymidine through skin and efficacy of different topical formulations against cutaneous herpes simplex virus infections in guinea pigs. *Antimicrob. Agents Chemother.* 26:819-823 (1984).
5. P.K. Wotton, B. Møllgaard, J. Hadgraft and A. Hoelgaard. Vehicle effects on topical drug delivery III. Effect of Azone on the cutaneous permeation of metronidazole and propylene glycol. *Int. J. Pharm.* 24:19-26 (1985).
6. N.V. Sheth, D.J. Freeman, W.I. Higuchi and S.L. Spruance. The influence of Azone, propylene glycol and polyethylene glycol on *in vitro* skin penetration of trifluorothymidine. *Int. J. Pharm.* 28:201-209 (1986).
7. M. Goodman and B.W. Barry. Differential Scanning Calorimetry (DSC) of human stratum corneum. Effect of Azone. *J. Pharm. Pharmacol.* 37:Suppl. 80P (1985).
8. G.D. Diana, U.J. Salvador, E.S. Zalay, P.M. Carabateas, G.L. Williams, J.C. Collins and F. Pancic. Antiviral Activity of Some β-Diketones. 2. Aryloxy Alkyl Diketones. *In Vitro* Activity against Both RNA and DNA Viruses. *J. Med. Chem.* 20:757-761 (1977).
9. D.P. Benziger, and J. Edelson. Disposition of Arildone, an Antiviral Agent, After Various Routes of Administration. *Antimicrob. Agents Chemother.* 22:475-481 (1982).
10. J.A. Bosso, S.L. Spruance, and G. Wenerstrom. Tolerance and Percutaneous Absorption of Topically Applied Arildone. *J. Clin. Pharmacol.* 25:95-99 (1985).
11. J.M. Douglas, Jr., F.N. Judson, M.L. Levin, J.A. Bosso, S.L. Spruance, J.M. Johnston, L. Corey, J.A. McMillan, L.B. Weiner, J.A. Frank, Jr. A placebo-controlled Trial of Topical 8% Arildone Cream Early in Recurrent Genital Herpes. *Antimicrob. Agents Chemother.* 29:464-467 (1986).
12. E.J. Baker. *Absorption of Drugs Across the Skin.* Ph.D. Thesis, University of Nottingham, United Kingdom (1987).
13. A.M. Kligman and E. Christophers. Preparation of isolated sheets of human stratum corneum. *Arch. Dermatol.* 88:702-705 (1963).
14. B.J. Poulsen, E. Young, V. Coquilla and M. Katz. Effect of topical vehicle composition on the *in vitro* release of fluocinolone acetonide and its acetate ester. *J. Pharm. Sci.* 57:928-933 (1968).
15. J. Hadgraft. Structure activity relationships and percutaneous absorption. *J. Control. Release.* 15:221-226 (1991).
16. G.L. Flynn, H. Dürrheim and W.I. Higuchi. Permeation of hairless mouse skin II: Membrane sectioning techniques and influence on alkanol permeabilities. *J. Pharm. Sci.* 70:52-56 (1981).
17. R.L. Bronaugh and R.F. Stewart. Methods for *in vitro* percutaneous absorption studies III: Hydrophobic Compounds. *J. Pharm. Sci.* 73:1255-1258 (1984).
18. R.L. Bronaugh and R.F. Stewart. Methods for *In Vitro* Percutaneous Absorption Studies V: Permeation through damaged skin. *J. Pharm. Sci.* 74:1062-1066 (1985).
19. G.S. Hawkins and W.G. Reifenrath. Influence of skin source, penetration cell fluid and partition coefficient on *in vitro* skin penetration. *J. Pharm. Sci.* 75:378-381 (1986).