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Effect of 1,4-cyclohexanediol on percutaneous absorption and penetration of azelaic acid

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ABSTRACT

The objective of this study is to investigate the effect of 1,4-cyclohexanediol as a retardant on the percutaneous absorption and penetration of azelaic acid. Hairless rat skin was mounted on Franz diffusion cells and treated with topical formulations containing solubilized azelaic acid with and without 1,4-cyclohexanediol. The skin was separated into stratum corneum and the deeper skin layers. The azelaic acid collected in receptor medium and each layer at the end of each time point was extracted and quantified. A significant decrease in flux across the skin suggests a penetration retardation effect of 1,4-cyclohexanediol (42.50 μg/cm^2/h in the presence of vs. 76.25 μg/cm^2/h in the absence of) at active loading level of 1.13 mg/cm^2. The penetration retardation effect was also observed at higher active loading level (2.82 mg/cm^2). Furthermore, presence of 1,4-cyclohexanediol in the topical formulation did not reduce the skin and epidermal retention of azelaic acid, suggesting its potential use in the development of superior topical formation for reducing potential systematic side effect while maintaining therapeutic efficiency.

1. Introduction

Rosacea is a chronic condition characterized by recurrent episodes of facial flushing, erythema, papules, pustules, and telangiectasia in a symmetrical, facial distribution (Esther et al., 2007). A number of compounds have been investigated for control and treatment of rosacea, including, for example, metronidazole and azelaic acid. Azelaic acid (1,7-heptanedicarboxylic acid, AZA) is a saturated, straight-chained C_9-dicarboxylic acid (Dermar et al., 1989) that has been reported to be the active pharmaceutical ingredient in a number of prescription drugs for the treatment of rosacea and acne.

Treatment of dermatological disorders relies on the ability of active agents to effectively penetrate the stratum corneum (SC) from applied formulations and reached the deeper skin layers such as epidermis and dermis (Cross and Roberts, 2000). At mean time, due to the local nature of dermatological disorders and potential systematic side effect of the applied active agents, ideal topical formulations would impart maximal skin retention and minimal skin penetration. For personal care applications, for example, sunscreens and bug repellents, minimizing potential systematic toxicity effect of applied chemicals is just as important as providing protection benefits.

Research in the area of permeation enhancement or retardation yields valuable insights into the structure–activity relationships of permeation enhancers as well as retardants. A number of studies have been carried out to minimize the systemic penetration by using chemical penetration retardants (Asbill and Michniak, 2000). It has been reported that the existence of certain compounds N-0915 of a similar structure to Azone which acted as drug retardants rather than enhancers. The mechanism of N-0915 may be expected to condense the skin lipids making them less permeable (Hadgraft et al., 1996). For example, phospholipids as penetration retardants apparently reduced the skin absorption of flurbiprofen (Fang et al., 2003). Oxazolidinones have the ability to retain the applied drugs in the skin layers, resulting in low systemic permeation (Rajadhyaksha and Pfister, 1996; Seth, 1999). The structures of oxazolidinones, e.g., 4-decyloxazolidin-2-one, are closely related to sphingosine and ceramide lipids, which are found naturally in the upper skin layers, which may explain skin retention effect of oxazolidinones. S,S-dimethyl-N-(benzenesulfonyl) iminosulfurane, S,S-dimethyl-N-(2-methoxycarbonylbenzenesulfonyl) iminosulfurane, and S,S-dimethyl-N-(4-chlorobenzenesulfonyl) iminosulfurane decreased the permeation of hydrocortisone significantly ($P < 0.05$) (Kim et al., 1999). Ideally, a penetration retardant should be chemically and pharmacologically inert, nontoxic, non-irritant, and non-allergenic.

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Table 1
Formulations containing solubilized azelaic acid.

Formulation	Ingredient (g/100 g)			
	Azelaic acid	Niacinamide	1,2-Hexanediol	1,4-Cyclohexanediol
F1	10.0	4.0	25.0	–
F2	10.0	4.0	25.0	1.0

It should have a rapid and reversible onset of action, be potent in low concentrations, compatible with the formulation ingredients and cosmetically acceptable (Chattaraj and Walker, 1995).

In this study, 1,4-cyclohexanediol as a penetration retardant was investigated. Its effect on percutaneous absorption and penetration of azelaic acid from topical formulations was studied in an *in vitro* skin model.

2. Materials and methods

2.1. Materials

Azelaic acid was purchased from ALFA AESAR (Ward Hill, MA, USA) and 1,4-cyclohexanediol was purchased from Sigma–Aldrich (St. Louis, MO, USA). 1,2-Hexanediol was purchased from Sabina Corporation (Piscataway, NJ, USA). Klucel® MF was obtained from Hercules, Inc. (Wilmington, DE, USA). All other reagents are of analytical grade.

2.2. Methods

2.2.1. Preparation of azelaic acid formulations

Azelaic acid formulation containing 1,4-cyclohexanediol was prepared as follows: 1,4-cyclohexanediol and niacinamide were dissolved in a solution of 1,2-hexanediol in water. Azelaic acid was dispersed in the above solution with a stirrer and the mixture was heated to 45 °C. The pH was adjusted to 4.9 ± 0.1 with triethanolamine. The stirring at 45 °C was continued until azelaic acid was dissolved. The solution was allowed to cool to room temperature. Klucel® MF (0.75%) was added to the cooled solution while stirring until the solution was gelled. The azelaic acid formulation without 1,4-cyclohexanediol was prepared similarly. The role of 1,2-hexanediol in the formulations is twofold: one is to enhance solubilization of azelaic acid in aqueous solution; the other is to serve as a moisturizing agent. Niacinamide is present in the formulations to provide anti-inflammation benefits. The final formulations are listed in Table 1.

2.3. Animals

Male hairless rats (30–40 days old) were purchased from Radiation Medicine Institute for Laboratory Animal Research, Chinese Academy of Medical Sciences, Tianjin, China. The abdominal skin was surgically removed from the animal, and subcutaneous fat was carefully cleaned. The skin samples were stored at −20 °C until use.

2.4. In vitro percutaneous absorption and penetration studies

The skin sample was mounted to Franz diffusion cells (diffusion area 1.77 cm^2) with SC side facing the donor chamber. The receptor chamber (16 ml) was filled with normal saline, which was continuously stirred with a magnetic stirrer setting at 500 r.p.m. The skin sample was equilibrated with normal saline for 1 h at 32 ± 0.1 °C. Finite doses (20 and 50 mg of the formulations, which correspond to 2 and 5 mg azelaic acid, respectively) were applied to the skin surface. Each experiment was run in six replicates. At the end of each time interval (8, 12, 16, 20, and 24 h), the skin surface was wiped with cotton ball soaked with phosphate buffered saline (pH 8.0). The tape-stripping method was used to remove the SC layer. The first strip was combined with the soaked cotton ball. The combination was digested with 1.0 M NaOH, neutralized to pH 5.0 using glacial acetic acid, filtered, and ready for HPLC analysis (Howes et al., 1996). Repeated tape-stripping (average 10 strips) was continued until SC layer was disappeared. All the strips were collected, combined, and digested in 10 ml 1.0 M NaOH. The mixture was then neutralized to pH 5.0 using glacial acetic acid, filtered, and ready for analysis. AZA retained within the epidermis/dermis layer was collected by methanol extraction. After tape-stripping, the remaining skin was minced, vertexed with 1 ml methanol and centrifuged, the supernatant was removed. The extraction step was repeated three times. The supernatants were combined, filtered and ready for analysis. The flux of AZA through the skin into the receptor fluid was determined from slopes of plots of concentration in the receptor phase vs. time and expressed as $\mu g/cm^2/h$.

2.5. HPLC analytical methods

HPLC analysis was carried out with an Agilent 1100 HPLC system. A 250 mm × 4.6 mm stainless steel C_{18} column (5 μm, Thermo, USA) was used. The mobile phase was a mixture of ammonium acetate/acetic acid buffered solution (pH 5.0, 10 mmol/L) and methanol at 60:40 (v/v) (Mansour and Ibrahiern, 2002). The flow rate was 1 ml/min. The ultraviolet detector was set at a wavelength of 210 nm and the temperature of the column was maintained at 30 °C.

2.6. Statistical analyses and data presentation

Statistical analyses were performed using Excel software. Paired two-tailed Student's *t*-test was performed to calculate the statistical significance. Values were given as mean ± SD.

Enhancement ratio (ER) for flux was calculated by using the following formula (Goodman and Barry, 1988):

$$\mathrm{ER} = \frac{\text{flux for formulation containing retardant}}{\text{flux for control formulation}}$$

3. Results and discussion

In this study, 1,4-cyclohexanediol as a potential penetration retardant was investigated in an *in vitro* skin model of hairless mouse. Hairless mouse skin tends to be thinner and has few layers in the SC. Therefore, the hairless mouse skin is more permeable than human skin (Catz and Friend, 1990; Fang et al., 2003). The hairless mouse skin is, perhaps, not a good membrane for screening skin penetration enhancers. However, it is quite suitable for studying the permeation retardation effect, precisely due to its lower permeation barrier. It is reasonable to assume that if a permeation retardation effect were observed in the more permeable mouse skin, it would have been more likely that a similar effect would be observed in the less permeable human skin.

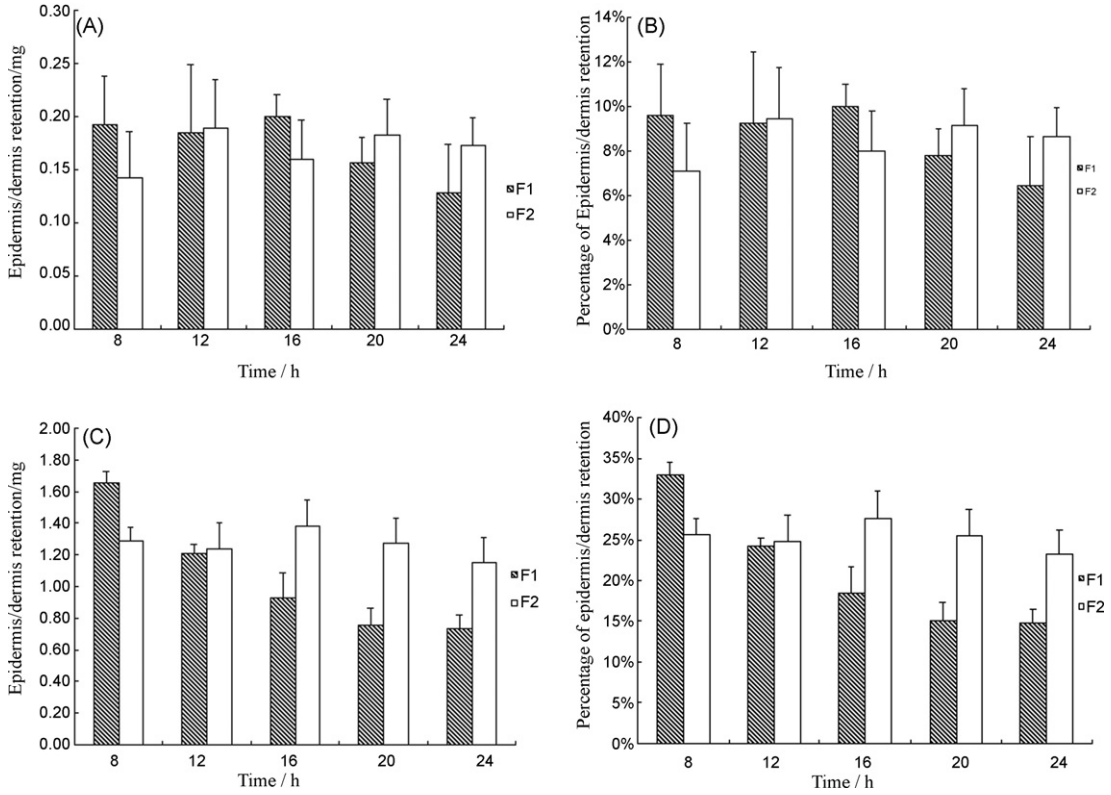

Fig. 1. (A) Epidermal retention of AZA (2 mg dose), mean ± SD, $n = 6$. (B) Percent of the applied dosage (2 mg dose), mean ± SD, $n = 6$. (C) Epidermal retention of AZA (5 mg dose), mean ± SD, $n = 6$. (D) Percent of the applied dosage (5 mg dose), mean ± SD, $n = 6$.

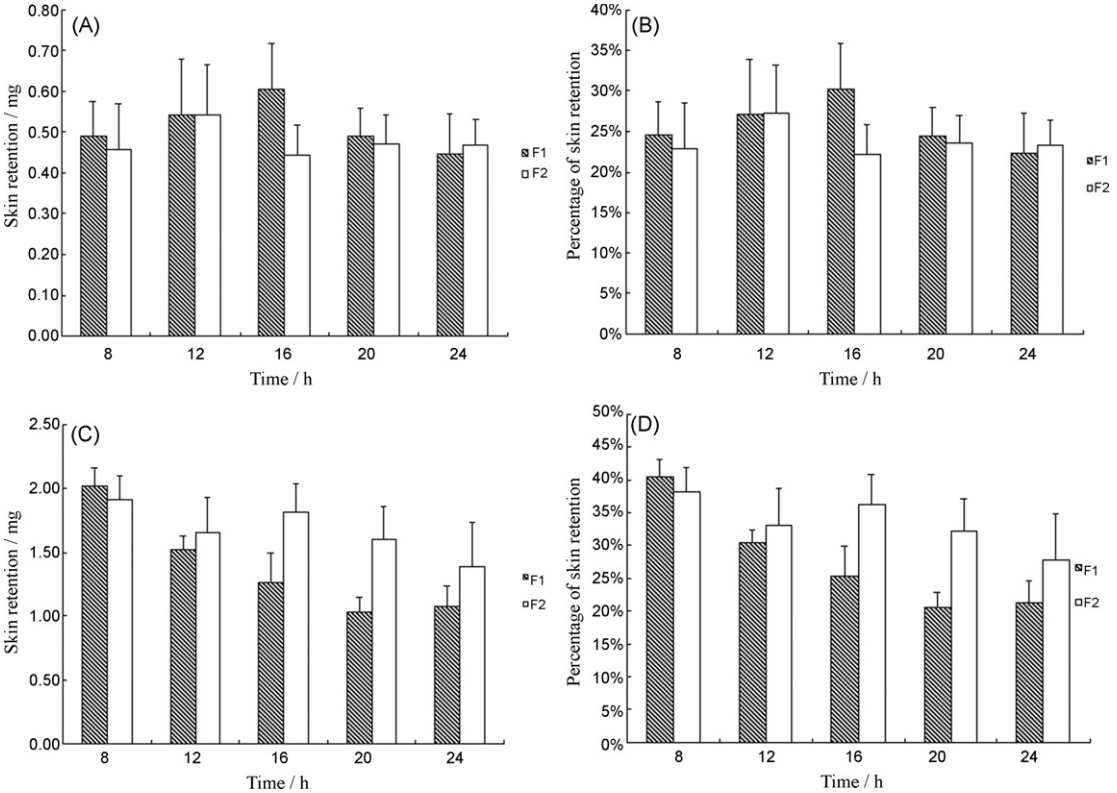

Fig. 2. (A) Total skin retention of AZA (2 mg dose), mean ± SD, $n = 6$. (B) Percent of the total skin retention (2 mg dose), mean ± SD, $n = 6$. (C) Total skin retention of AZA (5 mg dose), mean ± SD, $n = 6$. (D) Percent of the total skin retention (5 mg dose), mean ± SD, $n = 6$.

Table 2
Flux and amount of azelaic acid collected in receptor medium at 24 h after application.

Formulation	Dose	Flux (μg/cm²/h)	Amount in collection medium at 24 h (μg)	ER
F1	2 mg	76.25 ± 29.15	1465.19 ± 81.36	–
F2	2 mg	42.50 ± 5.20	1392.70 ± 131.19*	0.56
F1	5 mg	187.18 ± 52.84	3448.90 ± 187.18	–
F2	5 mg	141.95 ± 18.93	3233.46 ± 220.99*	0.76

Each value represented the mean ± SD (n = 6). ER, enhancement ratio.
* P;0.05.

3.1. Epidermal retention of azelaic acid

The retention of AZA in the epidermal layer from the two formulations as a function of time is shown in Fig. 1. For the 5 mg dosage, retention of azelaic acid in the epidermal layer for the formulation without 1,4-cyclohexanediol (formulation F1) decreased significantly from 1.65 ± 0.08 to 0.74 ± 0.08 mg during the 24 h period after administration. On the other hand, retention of azelaic acid in the epidermal layer for the formulation with 1,4-cyclohexanediol (formulation F2) remained steadily at about 1.2 mg over same period of time. These data suggest rapid depletion of azelaic acid for the formulation without 1,4-cyclohexanediol and steady absorption and penetration of azelaic acid for the formulation with 1,4-cyclohexanediol.

For the 2 mg dosage, although retention of azelaic acid in the epidermal layer was about the same for both samples, the formulation without 1,4-cyclohexanediol gave significantly higher flux value than with 1,4-cyclohexanediol (see discussion in Section 3.3).

These results suggest that the presence of 1,4-cyclohexanediol enhances retention of azelaic acid in epidermal layer and retards penetration of azelaic acid across the skin.

3.2. Skin retention of azelaic acid

Fig. 2 shows the total skin retention (sum of the SC and epidermal layers) of the two formulations at various time points. The data suggest a similar trend as observed in the case of epidermal retention and offer further evidence of the penetration retardation effect of 1,4-cyclohexanediol.

At the 5 mg dose, lower level of total skin retention and faster penetration was observed for the formulation without 1,4-cyclohexanediol than with 1,4-cyclohexanediol. At the 2 mg dose, although the level of total skin retention is similar for the formulations, the flux values (see Section 3.3) indicated much faster penetration for the formulation without 1,4-cyclohexanediol than with 1,4-cyclohexanediol.

3.3. Percutaneous absorption flux

As shown in Table 2, the flux value for the formulation with 1,4-cyclohexanediol (42.50 ± 5.20 and 141.95 ± 18.93) is significantly lower than without 1,4-cyclohexanediol (76.25 ± 29.15 and 187.18 ± 52.84). The effect was particularly profound for the 2 mg dose, where the flux was reduced by about 45% in the presence of the penetration retardant, 1,4-cyclohexanediol. Fig. 3 shows the amount of azelaic acid penetrating through the skin over a period of 24 h at different doses. Lower amounts of azelaic acid were collected in the receptor medium throughout all time points for the formulation without 1,4-cyclohexanediol.

The results showed that 1,4-cyclohexanediol as a penetration retardant markedly reduced the flux of azelaic acid across the skin, without significantly reducing the total skin and epidermal retention of azelaic acid. The flux data also suggest that 1,4-cyclohexanediol in the formulation makes the skin membrane less permeable, which is consistent with a skin barrier-controlled mechanism.

The results demonstrated that 1,4-cyclohexanediol is a penetration retardant for percutaneous absorption and penetration of azelaic acid. The penetration retardation effect of 1,4-cyclohexanediol may suggest its potential application in formulating topical formulations with improved therapeutic efficacy and reduced side effect. The results also indicated that the permeation process of solubilized AZA might be consistent with a skin-controlled mechanism, since the viscosity of formulations played a paramount role in controlling the release of the drug if the diffusion of drug through the matrix was a rate determining step (Ho et al., 1994; Bentley et al., 1999).

In terms of possible mechanism for retardant activity with 1,4-cyclohexanediol, we postulate that the effect might result from possible perturbation of skin lipid structure. In both formulations (with and without 1,4-cyclohexanediol), azelaic acid is fully solubilized. Therefore, it is less likely that the effect is due to a

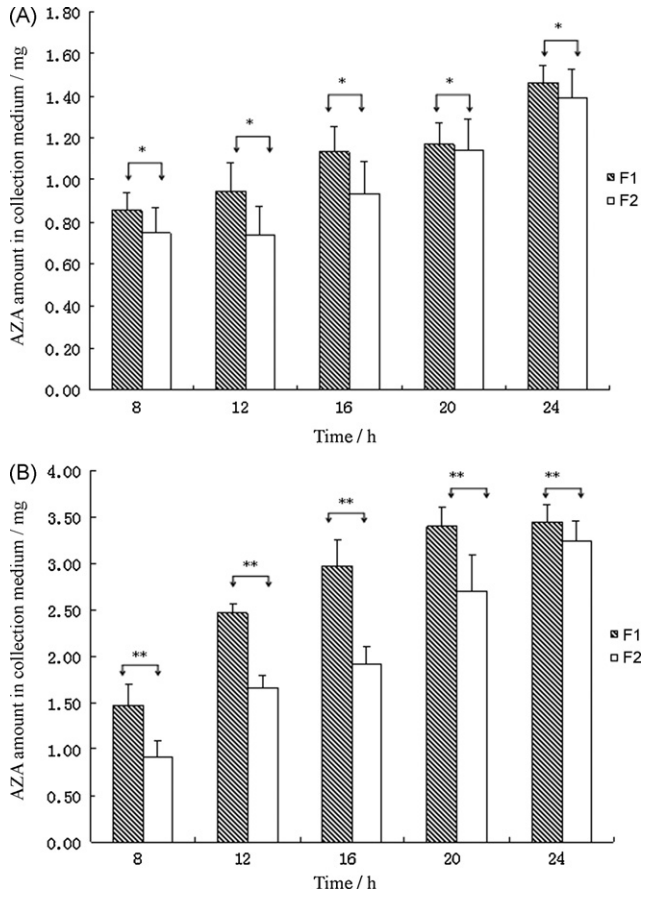

Fig. 3. (A) Amount of AZA in the receptor medium at various time points: comparison between formulations F1 and F2 (2 mg dose). Mean ± SD, n = 6. (B) Amount of AZA in the receptor medium at various time points: comparison between formulations F1 and F2 (5 mg dose). Mean ± SD, n = 6. *P < 0.05 and **P < 0.01.

solubilization or partitioning effect. Furthermore, generally speaking, carboxylic acids with long carbon chains do not form strong hydrogen bonds or complexes with alcohols in aqueous-based solutions. Thus, we postulate that interaction between azelaic acid and 1,4-cyclohexanediol would be relatively weak in both formulations. It is predicted that release of azelaic acid from both formulations would be the same with no skin present.

It has been reported that the hydrogen-bonding capability of a penetration modifying agent (enhancement or retardation) is a significant factor in determining its skin penetration effect (Abraham et al., 1995; Potts and Guy, 1995; Roberts et al., 1996). The most powerful hydrogen-bonding lipid in the SC layer is ceramide 6, which had four secondary alcohol and one secondary amide groups (Wertz, 1992). Thus, possible hydrogen-bonding interaction between 1,4-cyclohexanediol and the ceramide 6 facilitated by the unique chair-like structure of 1,4-cyclohexanediol and/or the chair-like structure itself might be responsible for the observed penetration retardation effect.

4. Conclusions

In this study, we investigated 1,4-cyclohexanediol as a penetration retardant for percutaneous absorption and penetration of azelaic acid in *in vitro* skin model. The presence of 1,4-cyclohexanediol in the formulations significantly reduced the flux of the applied azelaic acid without reducing the retention of azelaic acid in dermatologically relevant epidermal layer. This might suggest its potential use in dermatological formulations for reducing potential systematic side effect while maintaining therapeutic efficacy.

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References

Abraham, M.H., Chadha, H.S., Mitchell, R.C., 1995. The factors that influence skin penetration of solutes. J. Pharm. Pharmacol. 47, 8–16.

Asbill, C.S., Michniak, B.B., 2000. Percutaneous penetration enhancers: local versus transdermal activity. Pharm. Sci. Technol. Today 3, 36–41.

Bentley, L.B., Marchetti, J.H., Ricardo, N., Ali-Abi, Z., Collett, J.H., 1999. Influence of lecithin on some physical chemical properties of poloxamer gels: rheological microscopic and in vitro permeation studies. Int. J. Pharm. 193, 49–55.

Catz, P., Friend, D.R., 1990. Transdermal delivery of levonorgestrel. VIII. Effect of enhancers on rat skin, hairless mouse skin, hairless guinea pig skin, and human skin. Int. J. Pharm. 58, 93–102.

Chattaraj, S.C., Walker, R.B., 1995. Penetration enhancer classification. In: Smith, E.W., Maibach, H.I. (Eds.), Percutaneous Penetration Enhancers. CRC Press, Boca Raton, FL, pp. 5–20.

Cross, S.E., Roberts, M.S., 2000. The effect of occlusion on the epidermal penetration of parabens from a commercial test ointment, acetone and ethanol vehicles. J. Invest. Dermatol. 115, 914–918.

Dermar, M., Augusto, M., Rudolf, S., Constantin, E., 1989. Effects of azelaic acid on proliferation and ultrastructure of mouse keratinocytes in vitro. J. Invest. Dermatol. 93, 70–74.

Esther, J., Aditya, K.G., Melissa, D., Mark, G., Sally, H., 2007. Systematic review of rosacea treatments. J. Am. Acad. Dermatol. 56, 107–114.

Fang, J.Y., Hwang, T.L., Leu, Y.L., 2003. Effect of enhancers and retarders on percutaneous absorption of flurbiprofen from hydrogels. Int. J. Pharm. 250, 313–325.

Goodman, M., Barry, B.W., 1988. Action of penetration enhancers on human skin as assessed by the permeation of model drugs 5-fluorouracil and estradiol. I. Infinite dose technique. J. Invest. Dermatol. 91, 323–327.

Hadgraft, J., Peck, J., Williams, D.G., Pugh, W.J., Allan, G., 1996. Mechanisms of action of skin penetration enhancers/retarders: Azone and analogues. Int. J. Pharm. 141, 17–25.

Ho, H.O., Huang, F.C., Sokoloski, J.D., Sheu, M.T., 1994. The influence of cosolvents on the in-vitro percutaneous penetration of diclofenac sodium from a gel system. J. Pharm. Pharmacol. 46, 636–642.

Howes, D., Guy, R., Hadgraft, J., 1996. Methods for assessing percutaneous absorption. The report and recommendations of ECVAM Workshop 13. Altern. Lab. Anim. 24, 81–106.

Kim, N., El-Khalili, M., Henary, M.M., Strekowski, L., Michniak, B.B., 1999. Percutaneous penetration enhancement activity of aromatic S,S-dimethyliminosulfuranes. Int. J. Pharm. 187, 219–229.

Mansour, A.M., Ibrahiern, M.M., 2002. Simultaneous determination of azelaic and benzoic acids in topical preparations by liquid chromatography. J. Chromatogr. 55, 435–437.

Potts, R.O., Guy, R.H., 1995. A predictive algorithm for skin permeability: the effects of molecular size and hydrogen bond activity. Pharm. Res. 12, 1628–1633.

Rajadhyaksha, V., Pfister, W.R., 1996. Oxazolidinones. Drug Cosmet. Ind. 1, 36–47, 104–107.

Roberts, M.S., Pugh, W.J., Hadgraft, J., 1996. Epidermal permeability–penetrant structure relationships: 2. The effect of H-bonding groups in penetrants on their diffusion through the stratum corneum. Int. J. Pharm. 132, 23–32.

Seth, B., 1999. Transdermal delivery using decyloxazolidin-2-one. Arzneim.-Forsch. Drug Res. 42, 120–122.

Wertz, P.W., 1992. Epidermal lipids. In: Seminars in Dermatology, vol. 11, pp. 106–113.