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Influence of Vehicle Composition on the Penetration of Indomethacin through Guinea-Pig Skin

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In order to investigate the process of percutaneous absorption of indomethacin (IND) from aqueous ethanol solution, the flux of IND was determined under various conditions by using a specially designed diffusion cell which was attached to the back of a guinea-pig.

It was found that the flux of IND was governed by the initial concentration of IND, pH, the amount of ethanol in the vehicle and the amount of additive ester: the flux increased with increase in the concentrations of IND and added ester, but decreased with increase in pH and the amount of ethanol.

The interactions of the components of the vehicle with IND and the skin appeared to influence the process of the percutaneous absorption of IND very strongly.

Keywords——drug absorption; percutaneous absorption; anti-inflammatory drug; indomethacin; guinea-pig skin

Indomethacin (IND) has been one of the most extensively used nonsteroidal anti-inflammatory agents for oral or rectal administration. The therapeutic action of IND has been established in various diseases, such as rheumatoid arthritis, ankylosing spondylitis and osteoarthritis of large joints, but the gastrointestinal side effects of IND restrict its use in the treatment of these diseases.

In order to overcome this disadvantage, attempts have been made to find a suitable route of administration and much effort has been directed to the development of a topical dosage form having local activity without systemic toxicity. It was shown that IND was absorbed rapidly through guinea-pig and human skin when applied externally.[1] Furthermore, it was found that IND penetrated fairly deeply into the muscle after topical application to rat skin.[1a]

The penetration of drugs through the intact human skin has generally been shown to be very poor, as typically observed in the case of corticosteroids.[2] The composition of the vehicle is believed to affect significantly the ability of a drug in a topical formulation to penetrate the skin.[3] The release of a drug from the vehicle is expected to be influenced by drug-vehicle, drug-skin and vehicle-skin interactions.

Thus, the present study was undertaken on the mechanisms of penetration of IND across the epidermis, and in particular, on the influence of vehicle composition on the skin penetration of IND in relation to its physico-chemical properties.

Experimental

Materials——IND of J.P. grade was used. Diisopropyl adipate (DIPA, Van Dyk Co., U.S.A.), diethyl sebacate and isopropyl myristate (Nikko Chem. Co., Tokyo), octyl dodecyl myristate (Shinei Chem. Co., Osaka) and tributyrin (Nakarai Chem. Co., Kyoto) were used without further purification, since they all gave a single peak on gas chromatography with a 3% Silicone OV-17 column (Gasukuro Kogyo Co., Tokyo) at 170°.

Solubility Studies——Excess IND was added to a 10 ml tube containing McIlvaine's buffer or various esters. The tubes were shaken mechanically in a water bath at $35 \pm 0.1°$ for 12 hr, and the concentration of IND in the supernatant was determined spectrophotometrically at 320 nm with a Hitachi spectrophotometer, model 124.

Measurement of Acid Dissociation Constant (pK_a)——Values of pK_a of IND in aqueous solutions con-

taining various amounts of ethanol were determined by titration of a 0.4 mg/ml solution of IND with 0.01 N sodium hydroxide under a stream of N_2 gas.

Penetration through Guinea-Pig Skin——Male Hartley guinea-pigs (320—420 g), which had been shaved with an electric clipper one day before the experiment, were anesthetized by the intraperitoneal injection of a 25% (w/v) aqueous solution of urethane. A conical glass diffusion cell was attached to the back with α-cyanoacrylate, and 2 ml of the test solution of IND (the preparation of the test solution is described below) was added in the diffusion cell as shown in Fig. 1. The penetration experiments were carried out with either an open system or a closed system (*cf.* Fig. 1). After the penetration experiment, the IND in the vehicle solution was collected with *n*-propanol (designated as the sample solution), then the amount of IND was determined by high performance liquid chromatography as described below.

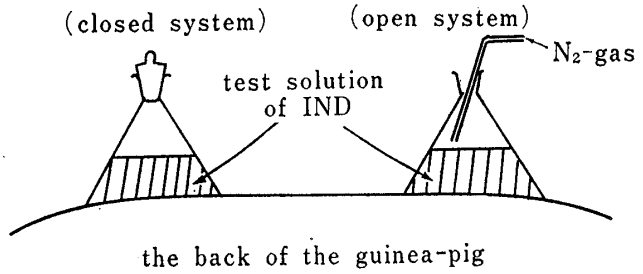

Fig. 1. Diagram of the IND Penetration Study on the Back of Guinea-Pigs

Preparation of the Test Solution——Accurately weighed IND was dissolved in the desired volume of ethanol, then distilled water was added to obtain a certain volume ratio of ethanol and water. During this process, diisopropanolamine or hydrochloric acid was added to keep the pH constant. Solutions containing various kinds of esters were prepared by adding suitable amounts of esters to the ethanolic solution of IND mentioned above.

Determination of IND——IND was determined chromatographically by the method of Skellern and Salole[4] with slight modifications: 1 ml of the sample solution was mixed with 8 ml of citrate buffer (pH 5.0) and 1 ml of an ethanol solution of flufenamic acid (1 mg/ml) as an internal standard. The IND in this mixture was extracted with 10 ml of *n*-heptane containing 3%(v/v) isoamyl alcohol. The heptane phase (8 ml) was evaporated to dryness under a stream of N_2 gas. The residue was dissolved in acetonitrile and the solution was injected onto the column (60 cm × 2 mm I.D.), which was packed with Bondapak C_{18}/Corasil (Waters Assoc., U.S.A.) and connected with a Waters Associates liquid chromatograph, model ALC 204. Elution was carried out with acetonitrile–0.1 M acetic acid (2: 3) at a rate of 1 ml/min.

Results

1. Effect of Ethanol in the Vehicle on the Penetration of IND

From the study on the solubility of IND at 35° in water, the solubility of IND was found to be about 1.0 μg/ml below pH 3, and it increased progressively as the pH was increased above pH 4 owing to the acid dissociation of IND (*cf.* Fig. 2). Though the solubility of IND in water increased with pH, it was still very low at pH 7 (403.2 μg/ml). The solubility of IND in water should be increased by the addition of ethanol, since the solubility of IND in ethanol is great (about 20 mg/ml). However, it is possible that ethanol affects the rate of percutaneous absorption of IND. Thus, the effect of ethanol on the permeation of IND through the back of guinea-pig was studied. The experiments were carried out by using a conical glass diffusion cell, as shown in Fig. 1, either in the closed system, where the concentration of ethanol was kept constant, or in the open system, where the concentration of ethanol became less as N_2 gas was introduced.

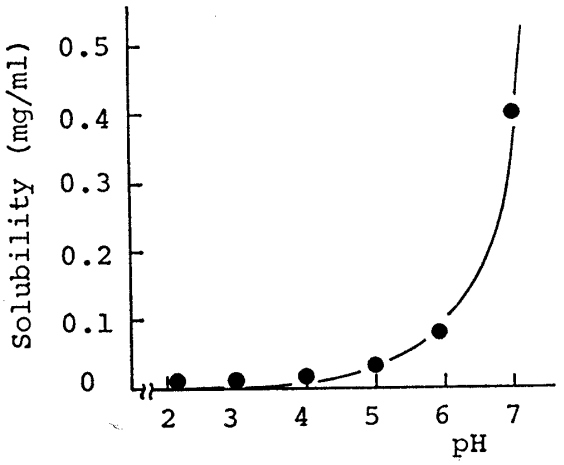

Fig. 2. Solubility of IND at Various pH Values

Fig. 3 shows the time course of the absorption of IND by the skin from 50% (v/v) ethanolic aqueous solution (initial concentration of IND; 0.5 mg/ml) with both systems. It is clear from Fig. 3 that the amount of IND absorbed was always greater in the open system than

in the closed system, and in 5 hr the amount absorbed in the open system was about 1.6 times greater than that in the closed system. It appears that the presence of a large amount of ethanol in the vehicle suppresses the penetration of IND through guinea-pig skin.

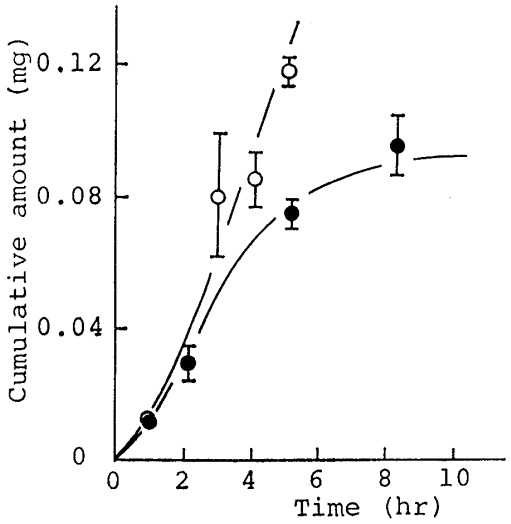

Fig. 3. Percutaneous Absorption of IND from 50% (v/v) Ethanol Solution

●: closed system, ○: open system.
The data are means of the results of five experiments and vertical bars are standard errors.

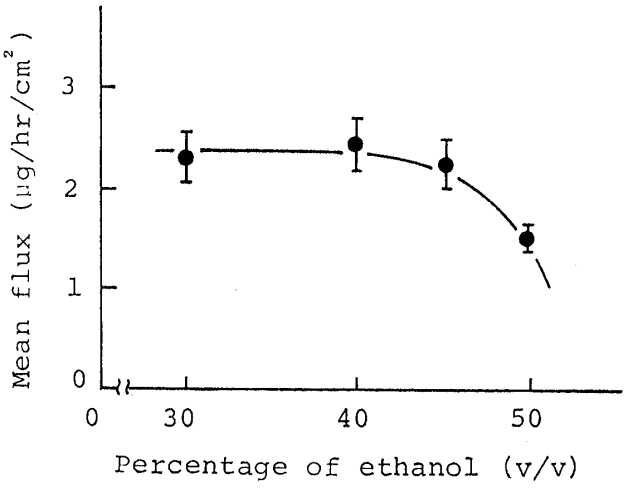

Fig. 4. Correlation between the Mean Flux and the Solubility of IND in Aqueous Ethanol Solutions

The data are means of the results of five experiments and vertical bars are standard errors.

To examine the effect of ethanol in the vehicle more precisely, the flux of IND through guinea-pig skin was determined with various amounts of ethanol in the vehicle from 30 to 50% (v/v) in the closed system (initial concentration of IND: 0.5 mg/ml). As shown in Fig. 4, the value of the mean flux of IND was about 2.4 μg/hr/cm² at 30 and 40% (v/v) ethanol, but it decreased to 1.5 μg/hr/cm² at 50% (v/v) ethanol.

For percutaneous absorption, two routes are possible: one is the transfollicular route and the other is the transepidermal route. As reported previously,[1a] it was found by microautoradiography that IND was absorbed through the epidermis when an ethanolic aqueous solution of ^{14}C-IND was applied to guinea-pig skin. The following equations were proposed to describe the process of drug penetration at equilibrium[5];

$$J = D_m \cdot K_m \cdot C_v / L \quad (1)$$

where J is the mean flux of a drug through the stratum corneum, D_m is the diffusion coefficient of a drug through the stratum corneum, K_m is the partition coefficient between vehicle and the stratum corneum, C_v is the concentration of a drug in the vehicle and L is the effective barrier thickness. Furthermore, Eq. (1) can be expressed as follows for various vehicle compositions:

$$J = D_m \cdot (C_v/S)/(L \cdot r) \quad (2)$$

where S is the solubility of a drug in the vehicle, r is the effective activity coefficient of a drug in the skin barrier and (C_v/S) is the so-called thermodynamic activity of a drug in the vehicle. In Eqs. (1) and (2), D_m, L and r are usually constant, so the changes of the flux (J) can be attributed to changes of the thermodynamic activity (expressed as C_v/S).

Shahi and Zatz[6] found that in the percutaneous absorption of hydrocortisone in propyleneglycol, the flux across rat skin increased linearly with the thermodynamic activity of hydrocortisone. However, this was not the case with IND, as shown in Fig. 5. A nonlinear

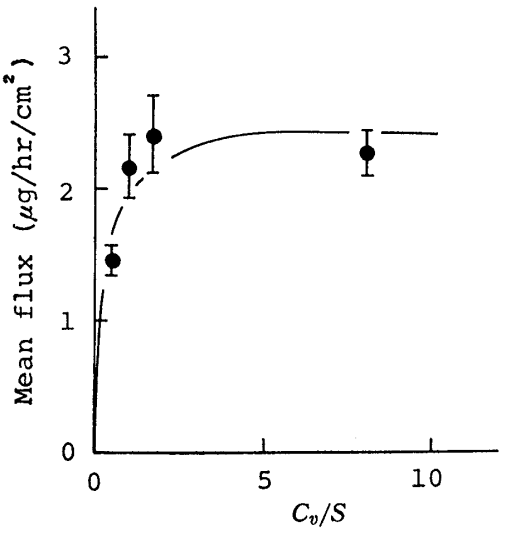

Fig. 5. The Mean Flux of IND as a Function of C_v/S

C_v: concentration of IND, S: solubility of IND. The data are means of the results of five experiments and vertical bars are standard errors.

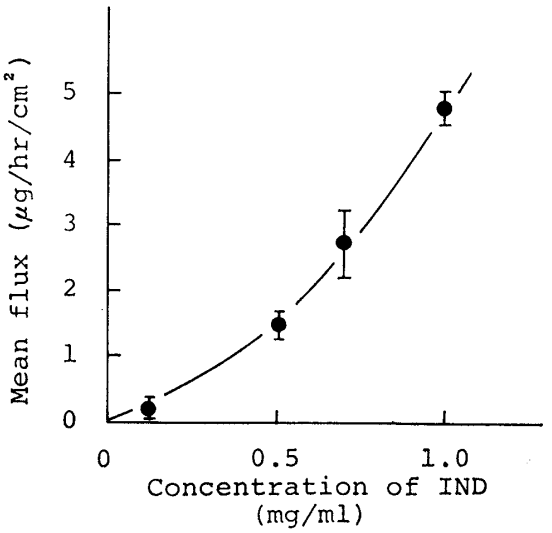

Fig. 6. Dependence of the Mean Flux on the Initial Concentration of IND

The data are means of the results of five experiments and vertical bars are standard errors.

relationship between the flux and the thermodynamic activity was also observed in the case of hydrocortisone dissolved in 2-propanol.[6] Since alcohols and hydrocarbons induce changes in the structure of biological membranes,[7] it is possible that alcohols, such as ethanol and propanol, in the vehicle change the properties of the skin, and this change influences the rate of absorption of IND.

2. Effect of IND Concentration on the Flux across the Skin

To see whether the flux of IND across guinea-pig skin depends on the concentration of IND in the vehicle, the flux was measured at various initial concentrations of IND from 0.1 to 1.0 mg/ml in 50% (v/v) ethanolic aqueous solution in the closed system. As shown in Fig. 6, the flux of IND depended on the initial concentration of IND, and the increase of the flux was not linear but curvilinear.

According to Eq. (1), a linear increase of the flux with the concentration of a drug should be observed, if the properties of the skin and the partition coefficient of a drug are constant during the penetration of a drug into the skin from the vehicle. However, the results in Fig. 6 show a discrepancy between the experimental results and the relation predicted by Eq. (1), as observed with some other anti-inflammatory agents.[8] The non-linear relation between the flux and the concentration of IND shown in Fig. 6 could be due to changes in the physiological properties of the skin, rather than to changes in the physico-chemical properties of IND, such as intermolecular association, since in the *in vitro* partition experiment no intermolecular association of IND was observed up to 2.8×10^{-3} M in the aqueous or organic phase.[9]

3. pH-Profile of the Flux

The acid dissociation of IND is expected to influence the process of penetration through skin layers, since the neutral form of a drug is generally transferred to the hydrophobic layer, such as cell membranes, much more effectively than the anionic form.[10] Thus, the mean flux of IND was determined from the amount absorbed by guinea-pig skin in 5 hr at various values of 50% (v/v) ethanol–H_2O in the closed system (initial concentration of IND: 0.5 mg/ml). In this case, the pH of the vehicle was adjusted to the desired value by adding diisopropanolamine or hydrochloric acid. As shown in Fig. 7, the flux decreased with increasing pH, being similar to the pH profile of acid dissociation of weak acids.

In the presence of 50% (v/v) ethanol, the pK_a of IND was determined to be 5.2, which coincides well with the value of 5.5 reported by Goto *et al.*[11] and that of 5.2 given by Fuwa *et al.*[12] Using the value of 5.2, the relative amount of the neutral form of IND at various pHs is shown in Fig. 7 as the fraction of unionized form of IND (dotted line in Fig. 7). Between pH 3 and pH 5, data points of the flux were located just on the curve of the fraction of unionized IND, indicating that only the neutral form of IND was transferred to the skin. However, at pH 6.2 the flux was greater than that expected from the relative amount of unionized IND, suggesting that at pH 6.2 the anionic form of IND was also transferred to the skin layer.

In a study of the partition mechanism of IND between *n*-octanol and water, we recently found that the anionic form of IND was also transferred to the hydrophobic region by forming an ion-pair complex with a cation in the aqueous phase,[9] as observed with 2,4-dinitrophenol[13] and methyl orange.[14]

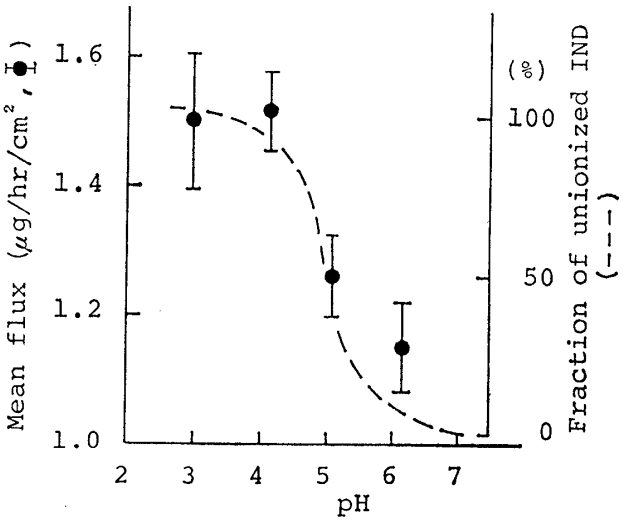

Fig. 7. Effect of pH on the Mean Flux of IND

The data are means of the results of five experiments and vertical bars are standard errors.

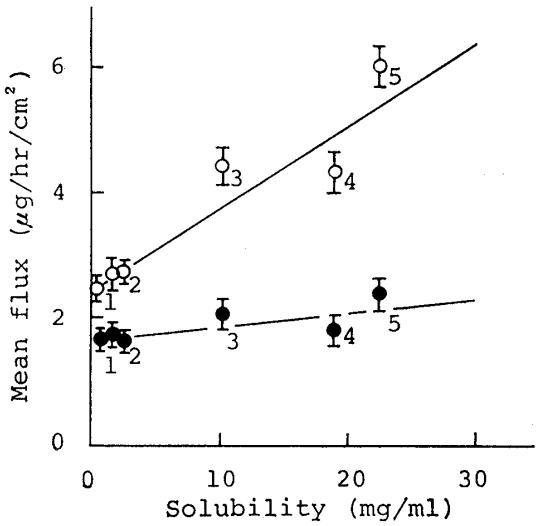

Fig. 8. Relationship between Mean Flux and Solubility of IND in the Presence of Various Esters

○: 40%(v/v) ethanol solution, ●: 50%(v/v) ethanol solution, 1: octyl dodecyl myristate, 2: isopropyl myristate, 3: tributyrin, 4: diisopropyl adipate, 5: diethyl sebacate.

The data are means of the results of five experiments and vertical bars are standard errors.

TABLE I. Solubility of IND in Various Esters

Number of carboxylate	Carbon number of carboxylate	Ester	Solubility (mg/ml)
Mono-	6	Ethyl butyrate	18.0
	8	Ethyl caproate	11.0
	12	Ethyl caprate	6.1
	14	Ethyl laurate	4.8
	16	Ethyl myristate	3.4
	17	Isopropyl myristate	1.9
	34	Octyl dodecyl myristate	1.1
Di-	8	Diethyl succinate	26.1
	10	Diethyl adipate	34.5
	12	Diisopropyl adipate	18.1
	14	Diethyl sebacate	22.9
Tri-	9	Triacetin	17.7
	15	Tributyrin	10.0

In view of these results, it is possible that the ion-pair complex of IND anion with diisopropanolamine as well as the neutral form of IND is responsible for the percutaneous absorption.

4. Effect of Esters on the Penetration Flux

Sometimes esters are added to the vehicle to maintain high solubility of hydrophobic drugs and to increase the effectiveness of topical application. Table I shows the solubility of IND in various esters at 35°. The solubility decreased as the carbon number of the monocarboxylate increased, and the di-carboxylate gave greater solubility than mono- and tri-carboxylate.

Next, the effects of various esters on the flux of IND were examined in the closed system. In 40 or 50% (v/v) ethanolic vehicle, various esters were added to a level of 1% (v/v) keeping the concentration of IND at 0.5 mg/ml. Fig. 8 shows the mean flux of IND in 5 hr as a function of the solubility of IND in esters. The fluxes of IND in the presence of 40 and 50% (v/v) ethanol increased as the solubility of IND in esters increased, and the flux in the presence of 40% (v/v) ethanol was always greater than that in the presence of 50% (v/v) ethanol, as expected from the results in Figs. 3 and 4. According to Eq. (2), the flux should decrease as the solubility of IND increases when the concentration of IND is kept constant. Thus, the effect of esters on the flux of IND could be due to the direct interaction of esters with the skin.

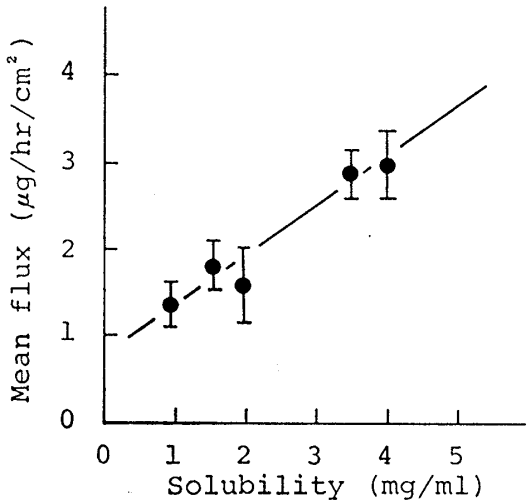

Fig. 9. Relationship between Mean Flux and Solubility of IND in the Presence of Various Amounts of DIPA

The data are means of the results of five experiments and vertical bars are standard errors.

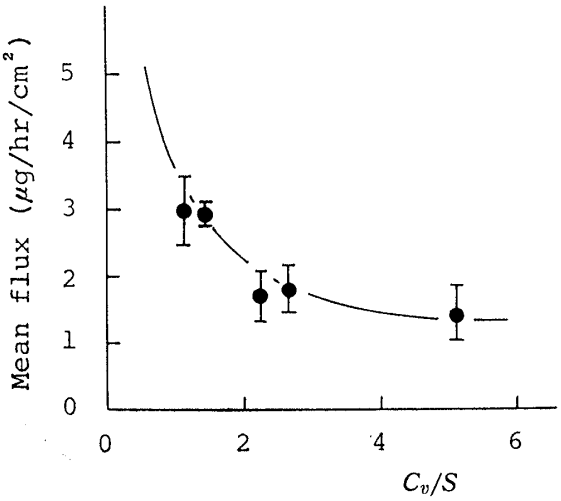

Fig. 10. Relationship between Mean Flux and C_v/S

The data are means of the results of five experiments and vertical bars are standard errors.

DIPA is commonly used as an additive in cosmetics and medical ointments, since DIPA is regarded as having no effect on the properties of the skin. Fig. 9 shows the changes in the penetration flux of IND with the concentration of DIPA (1—7%(v/v)) in 50% (v/v) ethanol–water. In the figure the flux is plotted as a function of the solubility of IND in the presence of DIPA. The flux increased almost linearly with increase in the solubility. The relation of the flux to the thermodynamic activity of IND expressed as C_v/S is shown in Fig. 10; the flux decreases as C_v/S increases, contrary to the prediction of Eq. (2). This result may indicate that the effect of DIPA on the flux is due to the interaction of DIPA with the stratum corneum: a change in the properties of the stratum corneum induced by DIPA enhances the penetration of IND.

Conclusion

In this study, the percutaneous absorption of IND was found to be influenced by the vehicle composition.

The concentration of IND in the vehicle affected the flux of IND, probably due to the direct interaction of IND with the skin. The absorption experiments at various pH values of the vehicle solution showed that unionized IND was absorbed more effectively than ionized IND, and that ion-pair complexes between the ionized IND and cations in the vehicle might be absorbed mainly through the stratum corneum at the region of pH one unit greater than the pK_a of IND. The flux of IND was influenced by ethanol and esters in the vehicle. These effects could not be explained completely on the basis of the changes in the thermodynamic activity of IND. Alteration of the physiological condition of the skin resulting from the interactions of the drug and the components of the vehicle with the skin also affected the results.

References and Notes

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