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Effect of ethanol on skin permeation of nonionized and ionized diclofenac

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Summary

The effect of ethanol on the skin permeation of diclofenac (DF) was investigated using excised hairless rat abdominal skin in vitro. The steady-state flux of DF increased with increase in the pH of DF-suspended donor solution; this phenomenon demonstrated a close correspondence with enhancement in the solubility of DF in the donor solution. In constrast, the steady-state permeability coefficient (P) of DF was inversely proportional to the change in pH of the donor solution, suggesting that the pattern of skin permeation of DF apparently obeyed the pH-partition theory, although the contribution of the ionized form of DF cannot be taken as being negligible. In order to determine the contribution of either the nonionized or ionized form on the skin permeation of DF, the permeability coefficients for each form (nonionized and ionized molecules) were calculated using the P values and the degree of ionization of DF in the donor solution. Addition of ethanol in the donor solution led to a marked decrease in the P value of nonionized DF, whereas the P value of ionized DF was not greatly affected by ethanol. A large amount of ethanol might increase the extent of permeation of DF through the lipid pathway by affecting the dense barrier structure of the skin. The flux of the ionized form of DF was particularly enhanced due to the increase in solubility as a result of the addition of ethanol, since the partition coefficient (skin/donor solution) of the ionized form was not greatly decreased compared with that of the nonionized form.

Introduction

Transdermal drug delivery has been recognized as an ideal route for the administration of drugs. Therefore, several studies have been performed with the objective of overcoming the low permeability of drugs through the skin. Ethanol is well known as an enhancer of transdermal drug delivery, and is therefore often formulated as a major component in some commercial ointments. Nishihata et al. (1988) have reported the promoting effect of ethanol on percutaneous absorption of diclofenac (DF). We have recently investigated the effect of cyclic monoterpenes on the percutaneous absorption of diclofenac sodium (DFS) from ethanol hydrogels in rats in vivo (Obata et al., 1992). It was observed that an increase in pH of the gel ointment led to the enhancement of percutaneous absorption of DF. Considering the

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pK_a value of DF in the gel ointment, ionized forms of DF were coexistent with nonionized forms in the gel ointment. Therefore, the contribution of ionized forms to the percutaneous absorption of DF may not be negligible.

In this paper, we investigated in vitro skin permeation of DF, employing two-chamber diffusion cells in which the abdominal skin of hairless rats was mounted. The contributions made by the nonionized and ionized forms of DF to the skin permeation of DF were determined. Furthermore, the promoting effects of ethanol on the permeability coefficients of the nonionized and ionized forms of DF were studied in detail, taking into consideration the change in pK_a values and solubilities of DF in the ethanol-buffer mixture.

Materials and Methods

Materials

DFS was generously supplied by SS Pharmaceutical Co., Ltd. DF was obtained by recrystallization of DFS in an acidic medium (0.1 N HCl solution). Other chemicals were of reagent grade.

Skin permeation study

Two-chamber diffusion cells (available diffusion area, 0.785 cm^2; volume of each half-cell, 3.0 ml) with a water jacket (37°C) were used (Tojo et al., 1987). Full-thickness abdominal skin was excised from male hairless rats (WBN rat, body weight 160–180 g) and mounted in the cells. The donor cell was filled with a suspension of DF (pH adjusted using McIlvaine buffer). Cosolvent mixtures were prepared by mixing ethanol and McIlvaine buffer in various ratios from 0 to 40% (w/w) ethanol. The receiver cell was filled with pH 7.2 phosphate buffer. Both cells were stirred using a magnetic stirrer. At appropriate intervals, 0.02 ml samples were taken from the receiver solution and replaced by the same volume of fresh buffer to maintain a constant volume. The concentration of DF in the samples was determined via HPLC. Permeation experiment was carried out until 8 h. After the lag time (within 2 h), plots of the cumulative amount of DF vs time showed sufficient linearity ($r \geq 0.95$).

Preparation of donor solutions

Excess amounts of DF (about 10-fold of solubility) were added to McIlvaine buffer or the cosolvent mixture of the buffer and ethanol. The ionic strength of each donor solution was made constant (0.5 M) using KCl (Elving et al., 1956) and the pH values of the drug suspension were again measured after being saturated. The drug suspension was transferred to the donor cell.

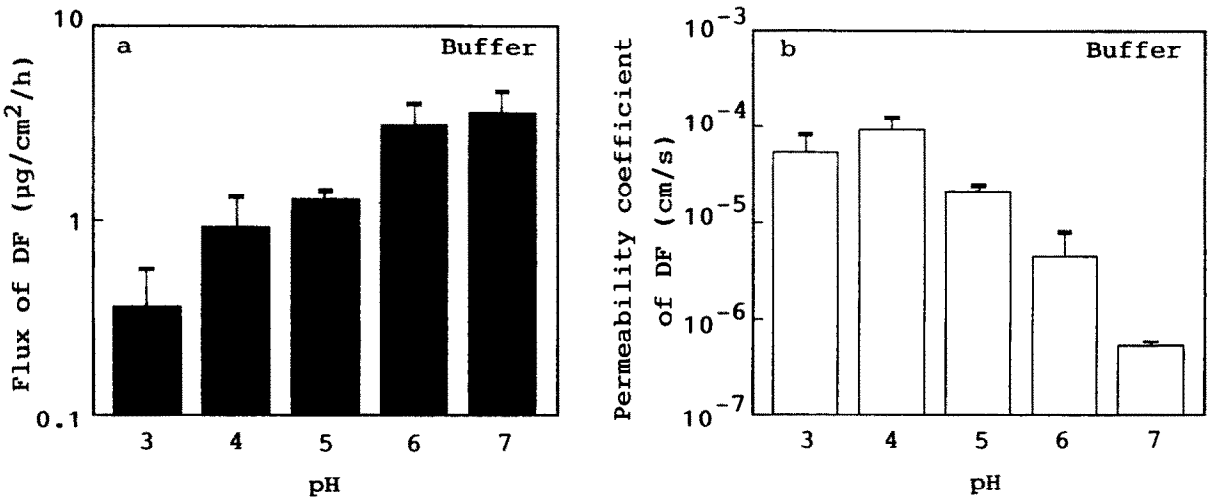

Fig. 1. Effect of pH on flux and permeability coefficient of DF in buffer system. Each column represents the mean ± S.D. for three determinations.

Determination of drug concentration

The sample solution (0.02 ml) in the skin permeation study was thoroughly mixed with methanol (0.2 ml) containing an appropriate amount of *p*-hydroxybenzoic acid *n*-hexyl ester as an internal standard. The mixture was filtered using a disposable filter unit (Gelman Science Japan Ltd, Ekikuro-Disk 3CR). The DF in samples was determined using HPLC apparatus (Model 655, Hitachi Ltd) equipped with a variable-wavelength UV monitor. The column was a YMC Packed A-302 S-5 120A ODS 4.6 × 150 mm (Yamamura Chemical Laboratories Co., Ltd). Elution was carried out at room temperature with a mobile phase consisting of 0.1% aqueous phosphoric acid-methanol (1 : 4 v/v) and the flow rate was 1.0 ml/min. The column effluent was monitored at 283 nm.

Determination of drug solubility

A DF suspension (including excess amounts of DF) prepared in the same way as described above (donor solution in the permeation study) was placed in a water bath (37°C) for 24 h under stirring with a magnetic stirrer. The sample was then filtered through a 0.45 μm membrane filter (Gelman Science Japan Ltd, Ekikurodisk 25CR). The concentration of DF was determined spectrophotometrically at 280 nm using a U-best 30 spectrophotometer (Japan Spectroscopic Co. Ltd; Tokyo, Japan).

Results and Discussion

Effect of pH on skin permeability of DF

The effect of pH of the donor solution on the steady-state flux of DF in the buffer system is shown in Fig. 1a. The steady-state flux of DF increased with increasing pH of DF-suspended donor solution. This phenomenon was consistent with our previous observation from in vivo experiments (Obata et al., 1992). The solubility of DF increased abruptly with increasing pH of the donor solution due to the increase in ionized forms of DF. Thus, the increase of flux in the higher pH region was considered to be brought about mainly by the increase in solubility of the ionized forms of DF in the donor solution. This result may suggest that the contribution of the ionized forms to the permeation of DF through the skin cannot be negligible. The steady-state permeability coefficient of DF in the buffer system is depicted in Fig. 1b. The maximum permeability coefficient was observed at around pH 3–4, suggesting that the charge on the skin surface was neutralized and that the lipophilicity of the skin might be maximized at these pH values (Katz and Poulsen, 1971). Further increase in pH of the donor solution led to a significant decrease in the permeability coefficient of DF. For instance, the permeability coefficient of DF at pH3 was about 100-fold greater than that of DF at pH 7. The pK_a value of DF has been reported to be 4.7 in aqueous solution at 25°C employing a titra-

TABLE 1

Solubility of DF (M)[a] *in media containing ethanol at various pH, and pK_a values estimated using the solubility data*

Ethanol (w/w%)	pH 2	pH 3	pH 4	pH 5	pH 6	pH 7	pK_a [b]
0	5.30×10^{-6}	5.89×10^{-6}	8.34×10^{-6}	5.11×10^{-5}	4.84×10^{-4}	5.90×10^{-3}	4.07
20	9.14×10^{-5}	9.14×10^{-5}	1.18×10^{-4}	4.62×10^{-4}	2.46×10^{-3}	3.46×10^{-2}	4.49
30	7.26×10^{-4}	7.32×10^{-4}	8.90×10^{-4}	1.79×10^{-3}	8.63×10^{-3}	9.44×10^{-2}	4.83
40	3.79×10^{-3}	4.77×10^{-3}	5.23×10^{-3}	8.92×10^{-3}	2.22×10^{-2}	9.98×10^{-2}	5.05

[a] Data are shown as the average of three determinations.

[b] pK_a values were calculated from Eqn 1 by using the solubility data at pH 2 as S_0.

tion method (Maitani et al., 1991). Therefore, DF was considered to be nonionized at pH 3 and ionized at pH 7. This may suggest that the skin permeation of DF was virtually explained based on pH-partition theory, although the contribution of the ionized form of DF cannot be neglected.

Effect of ethanol concentration on solubility of DF

At first, the pK_a values of DF in the cosolvent were determined on the basis of the solubility as follows:

$$pK_a = pH - \log\frac{S - S_0}{S_0} \quad (1)$$

where S is the solubility of DF at the current pH and S_0 denotes the solubility of the nonionized form of DF (which was determined at pH 2). The solubility data of DF in media containing ethanol at various pH values are summarized in Table 1. The pK_a values of DF were calculated from Eqn 1 by using the solubility data, and are also listed in Table 1. As shown in Fig. 2, the pK_a values of DF increased linearly with increasing ethanol concentration. This may suggest that the ratio of nonionized and ionized DF was greatly affected by the concentration of ethanol even though the pH was adjusted to the same value. The following equation for the pK_a values was obtained empirically as a function of ethanol concentration in the cosolvent with a significantly high value of the correlation coefficient ($r = 0.996$).

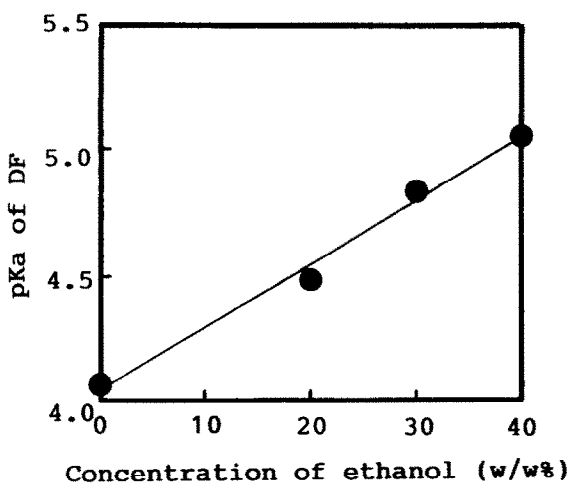

Fig. 2. pK_a values of DF determined at various concentration of ethanol.

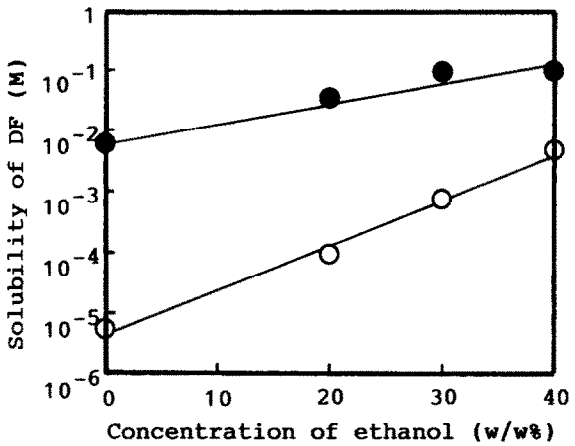

Fig. 3. Effect of ethanol on the solubility of the nonionized (S^n) and ionized (S^i) forms of DF. (○) S^n, (●) S^i. Slopes of solid line (σ value): 7.19, S^n; 3.28, S^i. Each point represents the mean of three determinations.

$$pK_a = 2.49f + 4.05 \quad (2)$$

where f is the weight fraction of ethanol in the cosolvent. From the result shown in Fig. 2, it is clear that the ratio of nonionized DF in the donor solution increases with increasing amount of ethanol.

In general, it is well recognized that cosolvents may increase drug solubility in solution. When the drug molecules exist in the nonionized form in the cosolvent, the solubility in the cosolvent (S_c) is often described as follows (Yalkowsky and Roseman, 1981):

$$\log S_c = \log S_w + \sigma f \quad (3)$$

where σ is a parameter representing the solubilizing power of the cosolvent and S_w denotes the solubility of drugs in a buffer solution. Fig. 3 demonstrates the solubility of the nonionized and ionized forms (S^n, S^i) as a function of ethanol in the cosolvent. Here, the S^n and S^i values were estimated from the pK_a values and solubility data listed in Table 1. In both the nonionized and ionized forms, the solubility of DF increased lin-

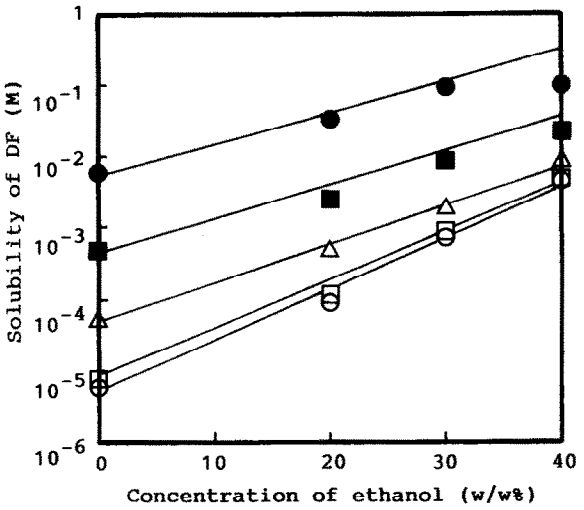

Fig. 4. Solubilities of DF in the cosolvent at various pH values. (○) pH 3, (□) pH 4, (△) pH 5, (■) pH 6, (●) pH 7. Each point represents the mean of three determinations. Solid line was obtained using Eqn 8.

early with increasing ethanol content, indicating that Eqn 3 was applicable not only to the nonionized form of DF but also to the ionized species. Values of 7.19 at S^n ($r = 0.994$) and 3.28 at S^i ($r = 0.948$) were respectively obtained for σ. Thus, the solubilities of the nonionized and ionized forms of DF in the cosolvent (S_c^n, S_c^i) were expressed as follows:

$$S_c^n = S_w^n \times 10^{7.19f} \tag{4}$$

$$S_c^i = S_w^i \times 10^{3.28f} \tag{5}$$

where S_w^n is the solubility of the nonionized form of DF in a buffer solution which is equal to that in the buffer solution at pH 2 (S_0). S_w^i denotes the solubility of the ionized form of DF in the buffer solution. From Eqn 1, the solubility of DF at the current pH in the buffer solution (S_w) is given as follows:

$$S_w = S_w^n(1 + 10^{pH - pK_a}) \tag{6}$$

where S_w^n corresponds to S_0 in Eqn 1. When Eqn 6 is applied for a cosolvent, the solubility of DF in the cosolvent (S_c) may be given as:

$$S_c = S_c^n(1 + 10^{pH - pK_a}) \tag{7}$$

S_c^n and pK_a can be substituted by Eqns 2 and 4, thus Eqn 7 is written as follows:

$$S_c = S_w^n \times 10^{7.19f}(1 + 10^{pH - 4.05 - 2.49f})$$

$$= S_0 \times 10^{7.19f}(1 + 10^{pH - 4.05 - 2.49f}) \tag{8}$$

The solubilities of DF in the cosolvents at various pH values were calculated using Eqn 8, and are plotted in Fig. 4. The experimental values of the solubilities corresponded well with those calculated, suggested that Eqn 7 can be applied for estimating the ratio of nonionized and ionized forms of DF in the cosolvent containing ethanol.

Effect of ethanol on flux and permeability coefficient of DF

The effect of ethanol added in the donor solution on the steady-state flux of DF was investi-

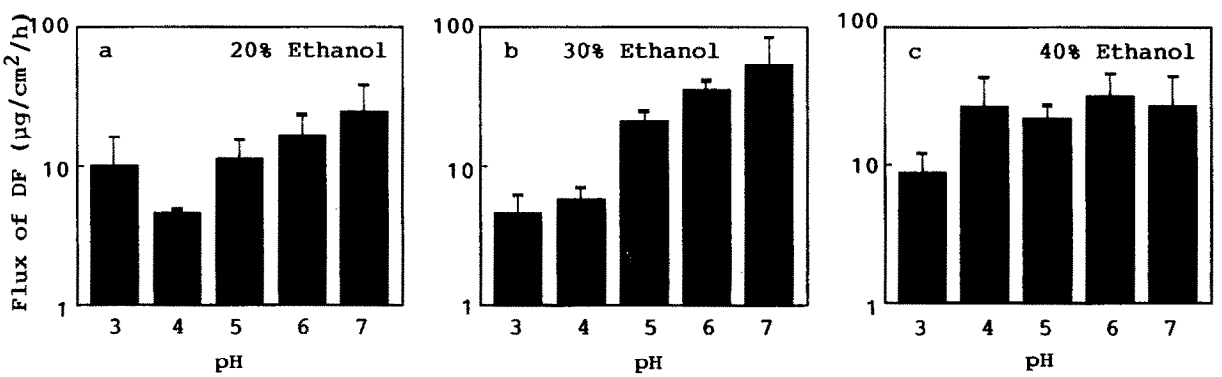

Fig. 5. Effect of pH on flux of DF in hairless rat skin at various concentration of ethanol. Each column represents the mean ± S.D. for three determinations.

gated over a wide range of pH in the donor solution. The results are shown in Fig. 5. The flux was approx. 10-fold greater compared with that observed in the buffer system at any pH value and at any concentration of ethanol. Fig. 6 depicts the permeability coefficient of DF obtained at the various concentrations of ethanol in the donor solution. At 20% ethanol (Fig. 6a), the permeability coefficient of DF was about 10-fold lower than that in the buffer system, suggesting that the addition of ethanol led to a decrease in the permeability coefficient. This decrease was inversely proportional to the increase in the solubility of DF resulting from the addition of ethanol in the donor solution. Thus, the increase in the DF flux is mainly caused by the increase in solubility of DF in the donor solution rather than the direct action of ethanol on the skin. The same tendency was observed when 30–40% ethanol was added in the donor solution (Fig. 6b,c), however, the effect of pH on the skin permeation of DF was weakened compared with that in the buffer or 20% ethanol systems.

In this study, the permeability coefficients of nonionized and ionized forms of DF were determined separately in order to clarify the effect of ethanol on the skin permeation of DF. The following equation can be derived under the assumption that the total flux was composed of the individual fluxes of the nonionized and ionized forms (Swarbrick et al., 1984).

$$J^t = P^n C^n + P^i C^i \tag{9}$$

where P^n and P^i are the permeability coefficients of the nonionized and ionized forms, respectively. C^n and C^i denote the concentrations of the nonionized and ionized forms in the donor solution, respectively. These values can be obtained from the Henderson-Hasselbalch equation as follows:

$$C^n = C^t - C^i \tag{10}$$

$$C^i = \frac{C^t}{1 + 10^{(pH - pK_a)}} \tag{11}$$

where C^t is a total concentration of DF in the donor solution. In this study, DF was suspended in the donor solution, therefore, the total solubility of DF (S^t) was used as the C^t value for estimating C^n and C^i values (i.e., S^n and S^i values). The permeability coefficients of nonionized and ionized DF were determined via Eqn 9 using the experimental values at pH 4 and 6. The results are shown in Fig. 7 with the solubility data for DF as a function of ethanol concentration. The permeability coefficient of the nonionized form was significantly lowered with increasing ethanol concentration in the donor solution. On the other hand, the permeability coefficient of the ionized form was not greatly affected by the addition of ethanol. In the case of the nonionized form of DF, the positive slope of the solubility relative to the concentration of ethanol was rather greater compared with the negative slope of the

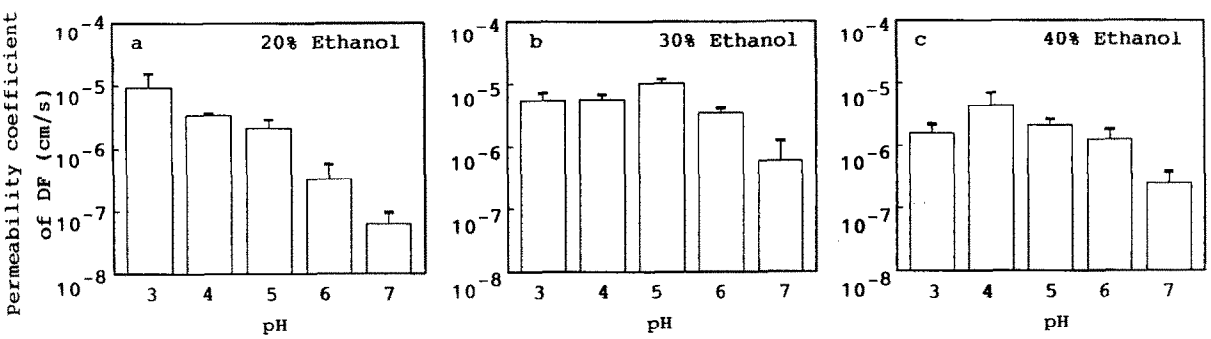

Fig. 6. Effect of pH on permeability coefficient of DF in hairless rat skin at various concentrations of ethanol. Each column represents the mean ± S.D. for three determinations.

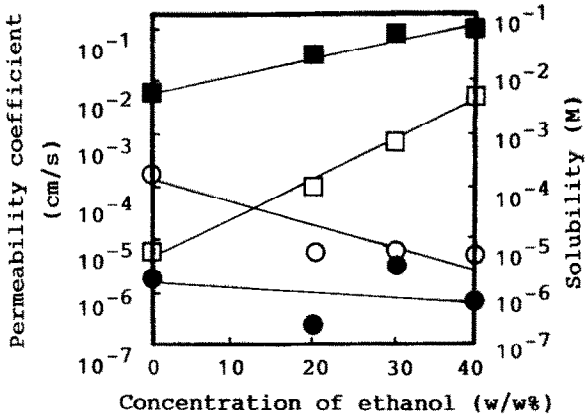

Fig. 7. Permeability coefficient (P) and solubility (S) of DF as a function of ethanol. (○) P^n, (●) P^i, (□) S^n, (■) S^i. Each point represents the mean of three determinations.

permeability coefficient, suggesting the direct action of ethanol on the skin.

Comparison between theoretical and experimental values of permeability coefficient

The partition coefficient (K_w) can be described as the ratio of the drug solubility in the skin (S_m) to that in the buffer (S_w):

$$K_w = \frac{S_m}{S_w} \tag{12}$$

In the case of the nonionized form of DF, the partition coefficient of the skin/cosolvent (K_f) can, by combining Eqns 4 and 12, be defined as;

$$K_f = \frac{S_m}{S_c^n} = \frac{S_m}{S_w^n} \times 10^{-7.19f} \tag{13}$$

If the cosolvent does not affect the drug solubility in the skin, the permeability coefficient of the nonionized form of DF (P^n) is expressed as:

$$P^n = \frac{K_f \times D}{h} = \frac{D}{h} \times \frac{S_m}{S_w^n} \times 10^{-7.19f} \tag{14}$$

where D is the diffusion coefficient, h denotes the effective length of diffusion, and the $(D/h \times S_m/S_w^n)$ value is equal to the experimental value of the permeability coefficient of nonionized form in buffer solution. Thus, the P^n values in the cosolvent may be estimated by using Eqn 14. If we assume ionized DF can also transport the lipid pathway in the skin (e.g., as an ion-pair), the permeability coefficient of the ionized form of DF (P^i) is expressed as:

$$P^i = \frac{D}{h} \times \frac{S_m}{S_w^i} \times 10^{-3.28f} \tag{15}$$

where the $(D/h \times S_m/S_w^i)$ value is equal to the experimental value of the permeability coefficient of ionized form in buffer solution. Then, the P^i values in cosolvent were also calculated in the same way as for P^n. The calculated and experimental values for the permeability coefficients are shown in Fig. 8.

In both the nonionized and ionized forms of DF, the experimental values of the permeability coefficient almost coincided with the theoretical values at low concentrations of ethanol (20%). However, a further increase in ethanol (30–40%) led to an upward deviation of the experimental data. The theoretical values were calculated based on the assumption that the skin was not altered by the cosolvent in the donor phase. Therefore, the results may suggest that the barrier structure of the skin was altered by a high concentration of ethanol such as 30–40%, although it was not

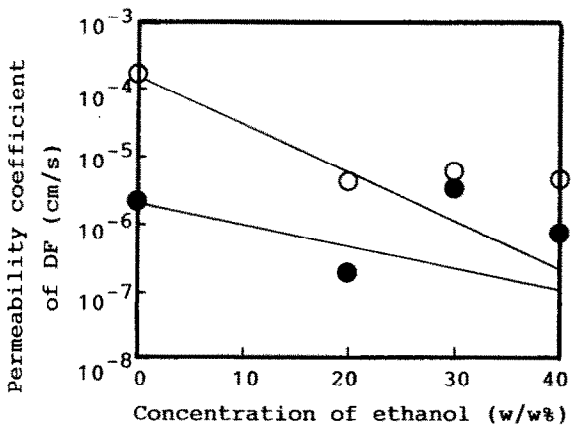

Fig. 8. Effect of ethanol on the permeability coefficient of DF. (○) P^n, (●) P^i. Each point represents the mean of three determinations. Solid lines: P^n and P^i values calculated by using Eqns 14 and 15, respectively.

greatly changed up to around 30% ethanol formulated in the donor solution.

Ghanem et al. (1987) have investigated the effect of ethanol on the permeation of β-estradiol, hydrocortisone, mannitol and tetraethylammonium bromide. As a result, it was reported that the permeability coefficient of lipophilic drugs such as β-estradiol was lowered by the addition of ethanol. This fact agreed well with the result observed with the nonionized form of DF as shown in Figs. 7 and 8. In contrast, the permeability coefficient of hydrophilic compounds such as mannitol and tetraethylammonium bromide was reported to be enhanced with increasing ethanol concentration. Kurihara-Bergstrom et al. (1990) also reported that the permeability coefficient of salicylate ion gradually increased as a function of ethanol concentration in the donor solution. The maximum permeability of salicylate ion was observed when 63% ethanol was formulated in the donor solution. If we assume that the ionized form of DF mainly permeates through the pore pathway in the skin and the cosolvent does not affect the structure of skin, the P^i value may not be affected by the partition coefficient of the skin/cosolvent. In this study, however, a decrease in the P^i value was observed with increasing solubility in the donor solution on the addition of ethanol, in analogy with the behavior of the P^n value, as shown in Fig. 8. Furthermore, the P^i value at 20% ethanol was close to the theoretical one calculated from Eqn 15 in which the lipid pathway was assumed as the route of the ionized form of DF. Thus, the permeation of the ionized form of DF through the lipid pathway as an ion-pair should be taken into consideration. In conclusion, a large amount of ethanol may increase the permeation of drugs through the lipid pathway by affecting the dense barrier structure of the skin. The flux of the ionized form of DF was especially enhanced by the addition of ethanol, since the partition coefficient of the ionized form was not markedly decreased compared with that of the nonionized form.

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