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The Influence of Cosolvents on the In-vitro Percutaneous Penetration of Diclofenac Sodium From a Gel System

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Abstract—The influence of cosolvents on the in-vitro percutaneous penetration of diclofenac sodium from a gel system was studied using a simplex lattice experimental design. Gel formulations were prepared by gelling the vehicle mixture of water, alcohol and propylene glycol with Carbomer 940. The synthetic membrane Durapore and hairless mouse skin were employed as barriers in a Franz-type diffusion cell. It was found that the penetration through the synthetic membrane was well described by the Higuchi model. There existed a better inverse relationship between the penetration rate and the drug solubility in the respective vehicle. It appeared to be a membrane-controlled mechanism when using hairless mouse skin as the barrier. The penetration rates in steady-state for nine formulations were fitted to a polynomial equation based on this simplex lattice method. A three-dimensional plot was constructed in this simplex surface studied. The maximal penetration rate was found to be from the vehicle containing water and ethanol in an exact volume ratio of 3:1 and the minimal penetration rate was observed from the vehicle containing water only.

Topical administration of therapeutic agents offers many advantages over oral and intravenous administration (Guy & Hadgraft 1985). However, the relative impermeability of the stratum corneum provides the principal resistance to the percutaneous penetration. Attempts using penetration enhancers, such as surfactant (Aungst et al 1986), organic solvents (Tsuzuki et al 1988), *N*-methyl 2-pyrrolidone and azone (Přiborský & Mühlbachova 1990), to reduce reversibly the resistance of this diffusion barrier have been reported. Cosolvents have been widely used as vehicles as well as penetration enhancers in the formulation of vehicle for topical drugs (Chiang et al 1989; Irwin et al 1990; Liu et al 1992). As well as regulating the ionization of the drugs, cosolvents may alter the barrier properties which in turn modify the transport profile. Thus, cosolvents used in the vehicle may have a profound influence on the percutaneous delivery from topical dosage forms, and provides a way to tailor the penetration rate of topical products by adjusting the ratio of cosolvents used.

Sodium diclofenac is a potent non-steroidal anti-inflammatory drug, used in the treatment of rheumatoid arthritis and other rheumatic disorders. It is extensively metabolized in the liver and mainly excreted in urine. Because of its short biological half-life, the drug has to be given frequently. As a result, developing a therapeutic system to provide transdermal delivery is beneficial. However, sodium diclofenac is not easily absorbed on transdermal application (Nishihata et al 1987). In this paper, the effect of cosolvents on the in-vitro penetration of diclofenac sodium through a synthetic membrane and the skin of hairless mouse from a gel dosage form is reported.

Materials and Methods

Materials

Diclofenac sodium was supplied by Yung-Shin Pharm. Co. (Taiwan). Triethanolamine and propylene glycol were obtained from Merck Co. Polyvinylidene difluoride (Durapore, type HVLP, 0·45 μm) was used as the synthetic membrane. Carbomer 940 was provided by BF Goodrich (Germany). Other reagents used were of analytical grade.

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Preparation of gel dosage forms

Gel dosage forms of diclofenac were prepared using a serial mixture of deionized water, propylene glycol and ethanol as the vehicle and a gelling agent of Carbomer 940 at a concentration of 1·0% w/w. In general, 1 g Carbomer 940 was dispersed in approximately 90 g cosolvent mixture initially. After complete hydration of Carbomer by the vehicle, 1 g drug was added and mixed to homogeneity. The mixture was gelified by the dropwise addition of 10% triethanolamine solution, and then the vehicle was added to give a total weight of 100 g. The pH value was determined after dissolving or dispersing 0·5 g gel in 50 g deionized water. The viscosity of the gel products was measured using a Brookfield Viscometer (model RV).

Solubility study

Excess diclofenac was added to the test vehicle and was agitated at 37°C for 24 h. The suspension was filtered through a membrane filter (0·45 μm) to obtain a clear solution. The concentration of diclofenac was measured by HPLC after appropriate dilution.

In-vitro penetration study

Hairless mice (strain ICR), age 6–10 weeks, ca, 20 g, were obtained from Veteran Memorial Hospital, Taipei, Taiwan. Mice were killed by spinal dislocation. Fresh skin was excised from the abdominal region, and was equilibrated in isotonic phosphate buffer (pH 7·2, 43 mM) before being placed on a Franz-type diffusion cell. Synthetic membranes were also soaked in the same medium for 24 h before mounting in the diffusion cell. The Franz-type diffusion cell was made of a

receptor compartment having a volume of about 5·5 mL and a donor compartment with an effective diffusional area of about 0·78 cm^2. About 2 g test gel product was placed on the donor side and isotonic phosphate buffer (pH 7·2, 43 mM) was used as the receptor medium, maintained at 37°C with jacketed circulating water and stirred at a constant rate of 400 rev min^{-1}. For skin, the dermis of the skin was in contact with the receiver compartment. At predetermined time intervals, 100 μL samples were withdrawn from the receiver compartment and an equal volume of fresh medium was added to maintain constant volume. Diclofenac was determined by HPLC.

Analytical procedure

HPLC analyses were undertaken using a system constructed from an Irica 871 pump, soma Detector-UV/vis/S and a reversed-phase column (15 cm × 4·6 mm i.d.) packed with Nucleosil (5 μm). Samples were injected in a volume of 20 μL and detection was at 276 nm. The mobile phase consisted of acetonitrile : 15 mM phosphate buffer (pH = 6·0) (40 : 60, v/v) and was operated at 1 mL min^{-1}.

Experimental design and kinetic calculations

A simplex lattice design was used to optimize the penetration rate by varying the volume of the three components water, propylene glycol and ethanol in the vehicle. Seven formulations were studied in a triangular space as shown in Fig. 1. These formulations included one at each vertex, one at halfway between the vertices, and one at the centre. One of the vertices was 100% water, and the other two were 50% w/w of water in propylene glycol or ethanol, respectively. With the restriction that the total weight must be equal to 500 g, both the active ingredient and Carbomer 940 were kept at 1% w/w. Replicates were run for the formulation at the centre point. The in-vitro penetration parameters were calculated from the penetration data using either equation 1 (skin) or equation 2 (synthetic membrane):

$$J_t = V/A \cdot dC/dt \quad (1)$$

$$Q_t = V/A \cdot C_t = \text{Constant} \cdot t^{1/2} \quad (2)$$

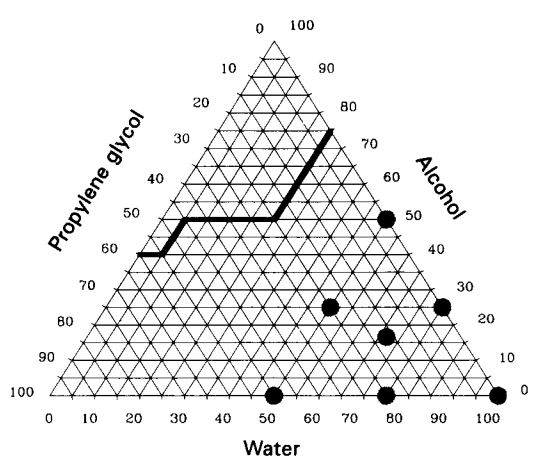

FIG. 1. Phase diagram for a three-compartment mixture. The region below the black thick line represents the vehicle mixtures giving a transparent gel and ● represent the formulations selected in the simplex lattice experimental design.

where J_t is the flux at time t, Q_t and C_t are the cumulative amount penetrated per unit area and the concentration in the receptor compartment at time t, respectively, V is the volume of the receiver compartment, A is the area available for penetration and dC/dt is the rate of change of the penetrant's concentration in the receiver side of the cell. Equation 1 was applied to the data collected during the steady-state period. All data are presented as the mean or the mean ± s.d. of at least five replicates. At steady-state, the flux (J_{ss}) through skin membrane is constant and is expressed by equation 3.

$$J_{ss} = D \cdot k \cdot \Delta C_s/h \quad (3)$$

where D is the diffusion coefficient in stratum corneum, k is partition coefficient between stratum corneum and the gel, h is the thickness of the stratum corneum, and $\Delta C_s/h$ is the concentration gradient across the stratum corneum. Because of the excellent solubility of diclofenac in the receptor fluid, sink conditions are maintained through the study. Hence the concentration gradient ($\Delta C_s/h$) can be considered equal to C_d/h, where C_d is the drug concentration in the gel:

$$J_{ss} = D \cdot k \cdot C_d/h \quad (4)$$

By definition, k is the ratio of drug solubility in stratum corneum (C_{sc}) to drug solubility in the gel (C_{gel}) so that

$$J_{ss} \cdot C_{gel} = (D/h \cdot C_{sc}) \cdot C_d \quad (5)$$

Based on equation 5, the enhancing effect of cosolvent on the penetration of drug can be separated from the effect of partition coefficient by normalization with respect to the drug solubility in the cosolvent (Dugard & Scott 1986). The enhancement factor of the cosolvent can be calculated by assuming $J_{ss} \cdot C_{gel}$ of formulation F1 equal to 1. The results are listed in Table 2.

Results and Discussion

The transport behaviour of diclofenac across the synthetic membrane or hairless mouse skin was investigated from a gel dosage form prepared by gelling a solvent mixture of water, propylene glycol and ethanol with Carbomer 940. The boundary (thick line) in the triangular phase diagram giving transparent gel was defined and the result is shown in Fig. 1 (the region below the thick line). A simplex lattice design was employed to optimize the solvent ratio with a desired penetration rate. This simplex is represented by a triangle within the region of the transparent gel as shown by Fig. 1. A total of nine formulations was selected including two replicates for formulation F7 (F8 and F9). The components of each formulation are listed in Table 1. Their physical properties (pH, viscosity, drug solubility in the corresponding vehicle) were measured and are listed in Table 1. Both F8 and F9 showed similar physical properties to that of F7 indicating reproducibility and reliability of the process.

Theoretically, the pH value of the vehicle, the drug solubility in the vehicle and the viscosity of gel matrix are three important factors to consider in the evaluation of drug penetration from a gel dosage form across the membrane or the skin. The pH value has an effect on the balance between ionized and un-ionized form of the drug, and ionized and un-ionized forms would show different penetration behaviour (Kushla & Zatz 1991). The viscosity of the gel matrix may

Table 1. Composition and physical properties of gel formulations.

	Formulation								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Composition (g)[a]									
Diclofenac	5	5	5	5	5	5	5	5	5
Carbomer 940	5	5	5	5	5	5	5	5	5
Alcohol	0 (0)	250 (100)	0 (0)	125 (50)	0 (0)	125 (50)	84 (33)	84 (33)	84 (33)
Propylene glycol	0 (0)	0 (0)	250 (100)	0 (0)	125 (50)	125 (50)	84 (33)	84 (33)	84 (33)
Distilled water	500 (100)	250 (0)	250 (0)	375 (50)	375 (50)	250 (0)	332 (34)	332 (34)	332 (33)
Properties									
pH value	7·6	7·2	7·0	7·2	7·4	6·8	7·0	7·0	6·9
Viscosity ($\times 10^{-3}$ cP s)	49·6[b] (1·2)	40·0 (1·5)	43·9 (0·6)	40·3 (1·5)	49·9 (2·1)	27·6 (1·0)	39·9 (1·5)	39·0 (1·9)	39·8 (1·3)
Solubility (mg mL^{-1})	34·1[b] (0·2)	95·5 (10·5)	145·4 (12·4)	75·1 (0·8)	59·1 (3·1)	121·7 (11·8)	90·2 (5·5)	92·9 (1·9)	95·8 (1·7)

[a] Transformed % values are in parentheses. [b] Mean (standard deviation), n = 5.

Table 2. Comparisons of the penetration rate through a synthetic membrane and hairless mouse skin.

Rate	F1	F2	F3	F4	F5	F6	F7	F8	F9
Membrane[a] (mg cm^{-2} h)	879·6 (94·3)	586·3 (42·9)	542·3 (53·6)	702·7 (53·2)	676·8 (73·3)	482·7 (18·8)	629·3 (22·2)	606·6 (43·7)	614·9 (53·6)
Mouse skin (mg cm^{-2} h)	18·88 (8·80)	40·38 (8·64)	29·25 (5·82)	52·64 (10·0)	44·05 (9·96)	21·36 (4·83)	34·25 (5·48)	30·19 (9·48)	25·22 (2·75)
$J_{ss} \cdot C_{gel}$	64·38	385·6	425·3	395·3	260·3	260·0	308·9	280·5	241·6
EF[b]	1	5·99	6·61	6·14	4·04	4·04	4·80	4·36	3·75

[a] Mean (standard deviation), n = 5. [b] EF = enhancement factor (calculated by assuming $J_{ss} \cdot C_{gel}$ of F1 = 1).

play an important role in controlling the release of the drug into the receiver compartment when the drug diffusion through the gel matrix is a rate determining step. The solubility of the drug in the vehicle will influence the partition coefficients of the drug between the gel and the membrane or the skin, in turn affecting the penetration rate of the drug. Cosolvents may also modify the structure of the skin, thus altering the penetration rate of the drug across the skin. All formulations were prepared with a similar pH value to minimize the pH effect. The effects of cosolvents on the penetration rate of the drug across the membrane or the skin were studied based on the difference of the viscosity and the solubility, or their influence on the skin structure.

As shown in Table 1, all formulations consistently had an aqueous pH value of around 7, but there were differences in viscosity and drug solubility. The viscosity of formulation F1 is the highest, containing only water as the vehicle and its drug solubility is the lowest. A lower viscosity was shown for those formulations containing both ethanol and propylene glycol (F6 and F7). Both ethanol and propylene glycol improve the drug solubility when added to the water. The solubility-enhancing ability is more powerful for propylene glycol than ethanol (F3).

The penetration of diclofenac from gel dosage form through the synthetic membrane Durapore was examined (Table 2, Fig. 2). When plotting the penetration rate vs square root of time as represented by equation 2, a linear relationship is obtained (Fig. 2) showing that the penetration of diclofenac across the synthetic membrane is well described by the Higuchi model, where the rate-controlling step is the process of diffusion through the gel matrix or the partition between two phases. However, a better correlation between the penetration rate and the solubility of the drug in the respective vehicle was found (Fig. 3), indicating that increasing the drug solubility in the vehicle slows down the penetration of the drug through the synthetic membrane. Thus, the partition of the drug between the membrane barrier and the gel matrix seems to play an important role in this process. The penetration of the drug through the synthetic membrane is via the pore structure within the membrane. The mean diameter of these pores is 0·45 μm and the pores are filled with receptor fluid, isotonic phosphate buffer. The pores are large enough for the drug molecules of either ionized or un-ionized form to diffuse freely. Therefore, the penetration of the drug might be simply dependent on the speed of drug partitioning from the gel matrix into receptor fluid.

When using hairless mouse skin as the barrier, the penetration rate is controlled by the skin membrane (Fig. 4). The penetration rates at the steady-state for the nine formulations were calculated based on equation 1 (Table 2). There was a larger deviation of penetration rate through the skin between formulation F7 and its replicates F8, F9 compared with the synthetic membrane. This larger deviation is probably due to the variation of mouse skin. Formulation F4 demonstrates the fastest penetration rate, whereas F1 shows the slowest.

This simplex design allows simple construction of a polynomial equation with seven terms that quantitatively fits the resulting data.

$$\text{Response} = b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 \quad (6)$$

where X_1, X_2, and X_3 represent transformed percentages for

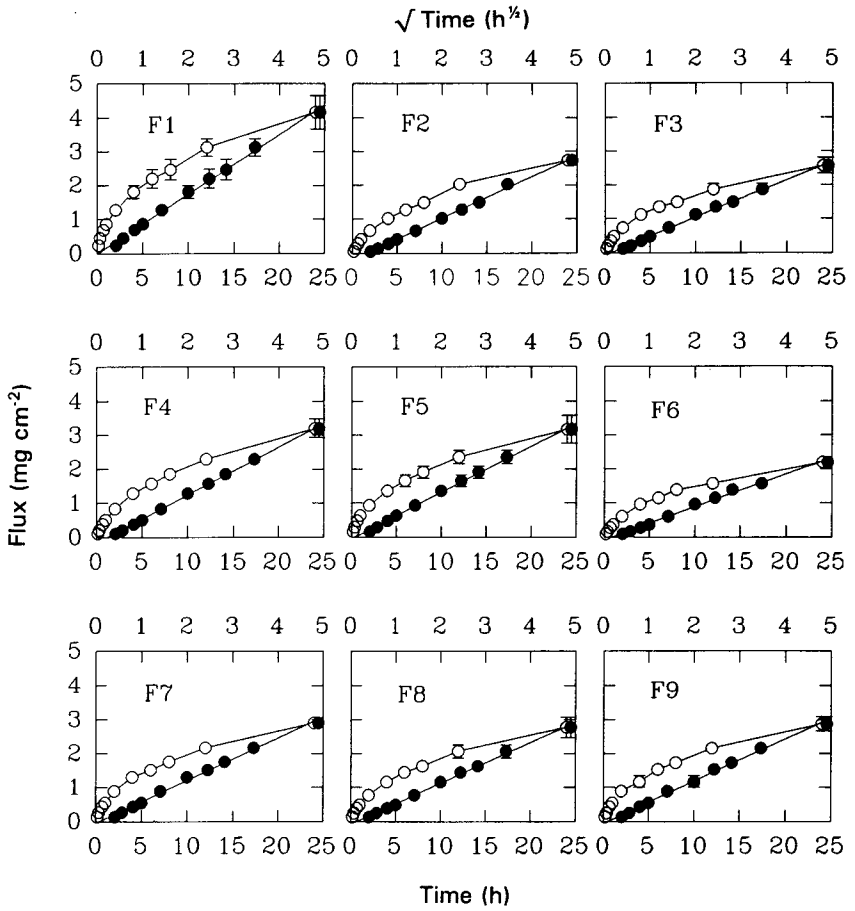

FIG. 2. Plots of the penetration rate vs time through the synthetic membrane (○) and the Higuchi relationship for the same data (●).

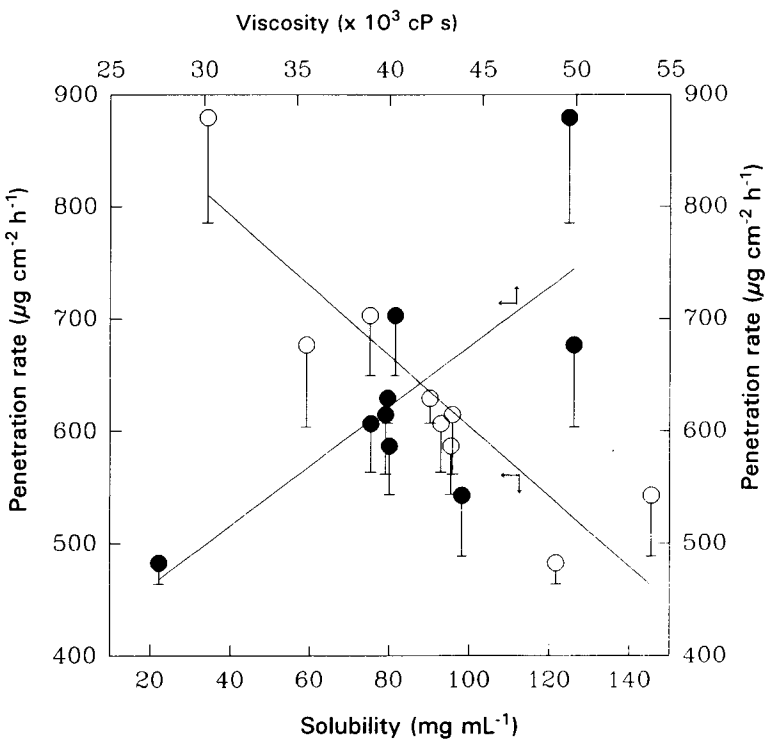

FIG. 3. Correlations between the penetration rate through the synthetic membrane and the viscosity (●) of the gel matrix or the drug solubility (○) in the respective vehicle.

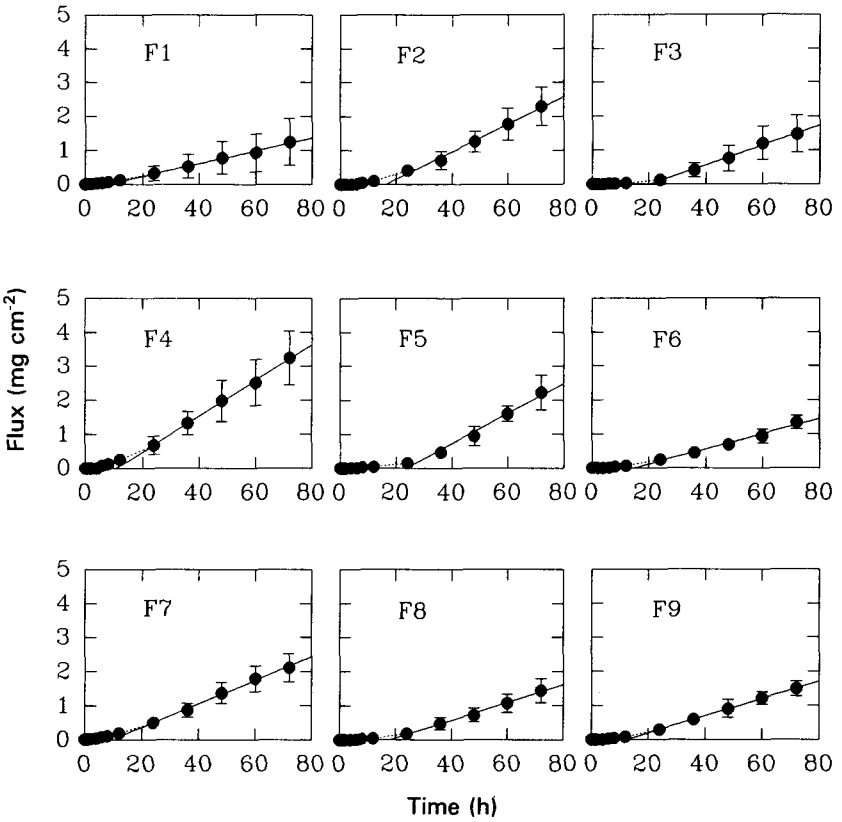

FIG. 4. Plots of the penetration rate vs time through hairless mouse skin.

water, alcohol, and propylene glycol, respectively. Using penetration rate as the response variable, the following response equation is obtained and should represent the response surface in the simplex space:

$$\text{Rate} = 18{\cdot}9\ X_1 + 40{\cdot}4\ X_2 + 29{\cdot}3\ X_3 + 92{\cdot}0\ X_1X_2 + \quad (7)$$

$$80{\cdot}0\ X_1X_3 - 53{\cdot}8\ X_2X_3 - 336{\cdot}1\ X_1X_2X_3$$

Fig. 5 illustrates two three-dimensional (3-D) plots viewed from two different angles of the penetration rate (z-axis) vs the percentage of water (x-axis) and alcohol (y-axis) in the vehicle based on this empirical equation. In a transformed water concentration of 0% (50% water concentration in the gel formulation) the penetration rate appears to decrease with decreasing alcohol concentration, minimizes near 60% transformed concentration of propylene glycol, and then increases at 100% transformed concentration of propylene glycol. In the absence of alcohol, the penetration rate increases with increasing propylene glycol concentration, maximizes near 55% transformed concentration of propylene glycol, and decreases at 100% transformed concentration of propylene glycol. Similarly, in the absence of propylene glycol, the penetration rate increases with increasing alcohol concentration, maximizes near 65% transformed concentration of alcohol, and decreases at 100% transformed concentration of alcohol. In this simplex surface studied, the maximal penetration rate is found to be from the vehicle containing water and ethanol in an exact volume ratio of 3:1 and the minimal penetration rate is observed from the vehicle containing water only.

An attempt to correlate the penetration rate through the skin with the drug solubility in the vehicle and the viscosity of the gel matrix was made. Neither factor showed a significant correlation with the penetration rate (Fig. 6). A membrane-controlled mechanism would mean that the hindrance for the drug to penetrate is the skin structure itself and the gel matrix would not be the rate-limiting step. As a result, the viscosity of the gel matrix shows only a minor influence on the drug penetration. However, if the skin is the main barrier for drug penetration, two factors influencing drug penetration through the skin should be considered. One is the partition of the drug between the gel matrix and the skin, and the other is the modification by the cosolvent of the skin structure. The former is a function of the drug solubility in the vehicle, and the latter is dependent on the various effects of cosolvent on the skin. As described above, there exists no significant linear correlation between the penetration rate and the drug solubility; therefore, the modifications of cosolvent on the skin should be considered.

Both alcohol and propylene glycol are common cosolvents reported to enhance drug permeation. The enhancing effect of alcohol on the permeation of an ionic molecule, salicylate, in human skin has been studied in an alcohol/water system (Kurihara-Bergstrom et al 1990). In their study, the enhancing effect of alcohol on salicylate ion reaches a maximum near 63% alcohol and then decreases as the ethanol concentration is further increased, which is similar to our findings with diclofenac. The proposal of a combination of alterations of protein conformation, reorganization within the lipid polar head regions, or lipid extraction within the polar

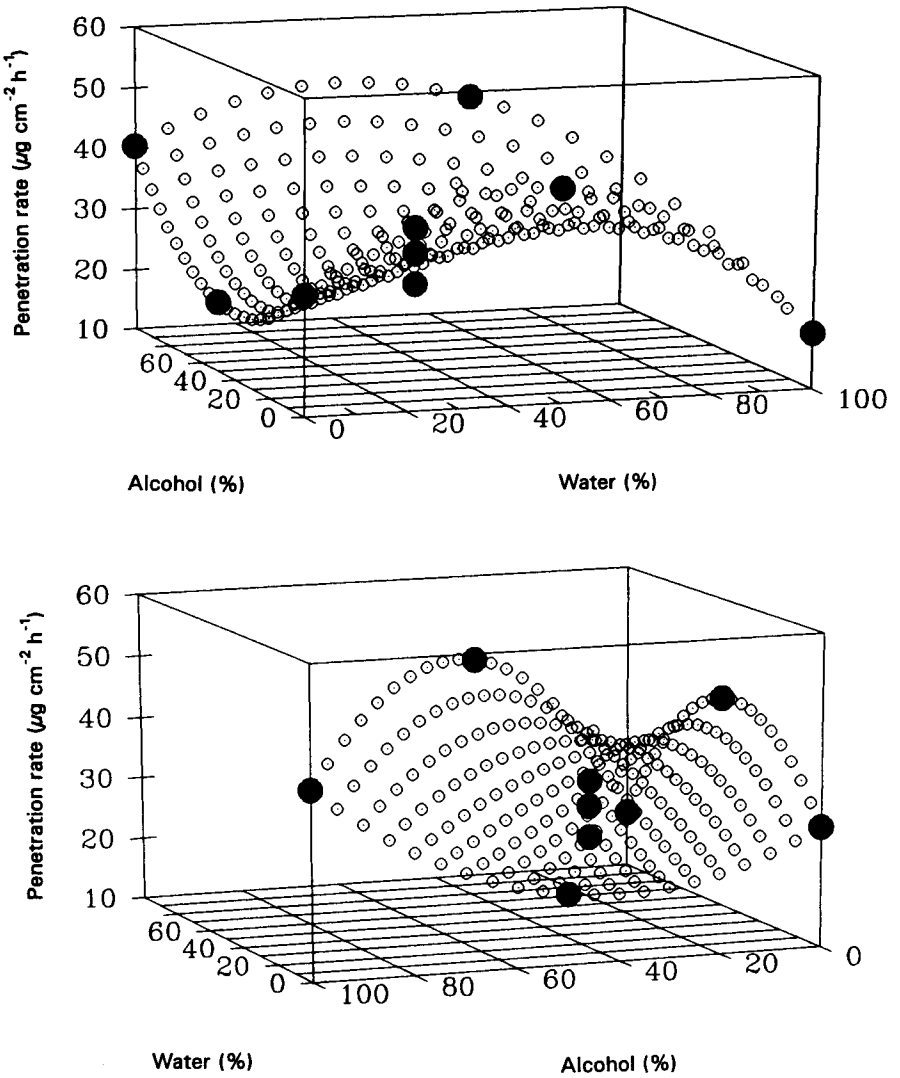

FIG. 5. Three-dimensional plot of the penetration rate through hairless mouse skin vs the vehicle composition based on the empirical equation obtained from a simplex lattic experimental design.

pathway to increase permeation with increasing alcohol content would also be valid in our case. The possible formation of ion pairs may further increase permeation as the ethanol concentration is increased. However, the increasing solubility with increasing alcohol concentration in the vehicle would hinder the partition of the drug into the stratum corneum. Therefore, these competing processes with increasing alcohol content would work for diclofenac resulting in optimal permeation near an exact alcohol volume fraction of 0·25.

It has been reported that the effect of propylene glycol is most noticeable when used in the in-vivo model where the stratum corneum is not fully hydrated (Barry 1991). A possible mechanism of solvating α-keratin and occupying hydrogen-bonding sites, thus reducing drug/tissue binding for propylene glycol is suggested. Thus, the enhancing effect of propylene glycol through alteration of the skin structure would increase with increasing the propylene glycol concentration in the vehicle mixture of water and propylene glycol. However, increasing the fraction of propylene glycol also increases the solubility of diclofenac in the vehicle mixture resulting in less partition of the drug into the skin. Therefore, two competing processes would operate simultaneously allowing a maximal permeation rate around an exact propylene glycol volume fraction of 0·25.

A minimal permeation of diclofenac was found for those vehicle mixtures containing a constant volume of water with various ratios of alcohol to propylene glycol. Since the enhancing effect of alcohol on the drug solubility is lower than that of propylene glycol, the replacement of alcohol with propylene glycol would cause less drug partitioning into the skin, thus reducing penetration rate. However, further increasing the fraction of propylene glycol increases the penetration rate gradually, probably due to alteration by propylene glycol of the skin structure promoting the penetration of the drug.

Overall, the influence of cosolvent on the penetration of drug can be considered as the simultaneous effects on partition coefficient and skin structure. As shown in Table 2, the enhancement factor, EF, of F2 changes little (from 5·99

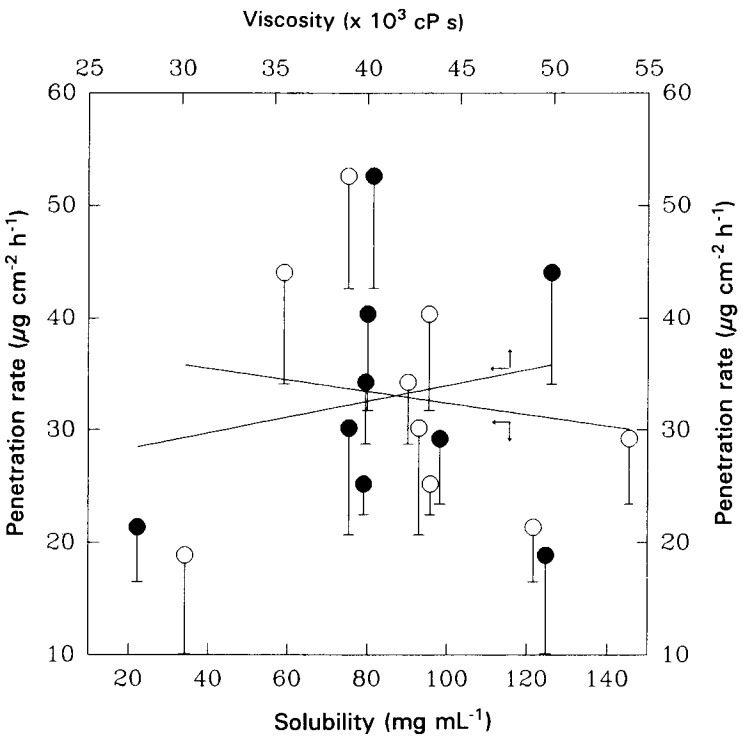

FIG. 6. Correlations between the penetration rate through hairless mouse skin and the viscosity (●) of the gel matrix or the drug solubility (○) in the respective vehicle.

to 6·14) when the exact volume fraction of alcohol decreases from 1:2 (F2) to 1:3 (F4). This indicates that alcohol may exert a similar effect on skin structure at these two volume fractions. However, when the exact volume fraction of propylene glycol increases from 1:3 (F5) to 1:2 (F3), the EF value increases from 4·04 to 6·61, suggesting that the effect of propylene glycol on skin structure increases with increasing volume fraction of propylene glycol. It also suggests that the effect of propylene glycol on skin structure is more profound compared with that of alcohol at the same volume fraction. According to equation 5, EF is a function of diffusion coefficient (D), thickness of stratum corneum (h), and drug solubility in stratum corneum (C_{sc}) assuming drug concentration in the gel (C_d) is constant during steady-state. The penetration of cosolvent into the stratum corneum is necessary for the cosolvent to exert its effects on skin structure. Simultaneous alteration by cosolvents on these three parameters is possible and the overall effect of cosolvent on the EF would be determined by the individual effect on each parameter. On the other hand, if alcohol or propylene glycol exert their effects by direct incorporation into the skin structure, the amount of the two cosolvents penetrating into the skin would determine the extent of their effects.

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