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Studies on the In-vitro Percutaneous Penetration of Indomethacin from Gel Systems in Hairless Mice

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Abstract

The influence of co-solvents on the in-vitro percutaneous penetration of indomethacin from gel systems was studied using a simplex lattice experimental design.

Gel formulations were prepared by gelling the vehicle mixture of water, either alcohol or isopropanol and either propylene glycol or PEG 400 with 1% w/w Carbomer 940. Hairless mouse skin was employed as the barrier in a Franz-type diffusion cell. The penetration rates at steady state for seven formulations were fitted to a polynomial equation based on this simple lattice method and a three-dimensional plot was constructed. The formulation having the maximal penetration rate was determined to be the vehicle with a solvent ratio of water : alcohol : propylene glycol equal to 15 : 33 : 52, and which possessed a solubility parameter of 15 and a drug solubility of around 10 mg mL^{-1}. When the solubility parameter of the vehicle was > 15, the drug solubility increased. However, the penetration rate decreased with an increasing solubility parameter. For those vehicles with a solubility parameter < 15, both the drug solubility and the penetration rate decreased with a decrease in the solubility parameter.

There was shown to be an approximately 20-fold increase in the relative enhancement factor when using both alcohol and isopropanol, but only a threefold increase for both propylene glycol and PEG 400, when compared with water.

Indomethacin is a non-steroidal anti-rheumatic agent which has a potent anti-inflammatory action but undesirable side-effects on the central nervous system. Furthermore, anti-rheumatic drugs of this kind also produce secondary side-effects on the stomach. The secondary side-effects from treatment with indomethacin orally have resulted in up to 20% of patients discontinuing therapy. As a result, developing a therapeutic system to provide a transdermal delivery would be beneficial. Without doubt, topical administration of therapeutic agents offers many advantages over oral and intravenous administrations (Guy & Hadgraft 1985); however, the stratum corneum provides the principal resistance to percutaneous penetration because of its relatively low permeability. Several attempts at using penetration enhancers, such as surfactants (Aungst et al 1986), organic solvents (Tsuzuki et al 1988), *N*-methyl 2-pyrrolidone and azone (Příborský & Mühlbachová 1990), to reversibly reduce the resistance of this diffusion barrier have been reported.

Recently, the use of cyclic monoterpenes, such as (+)-limonene and (−)-menthol, to enhance the percutaneous absorption of indomethacin was reported (Katayama et al 1992). Significant synergism of (+)-limonene with ethanol was also observed in the enhancement of percutaneous absorption of indomethacin (Takayama et al 1991). In addition, a possible mechanism of action for this binary enhancer system was proposed (Kikuchi et al 1992), which clearly indicates that the activities of penetration enhancers may also depend on the choice of the co-solvent mixture in such a gel system.

Co-solvents have been widely used both as the vehicle as well as the penetration enhancer in the formulation of vehicles for topical drugs (Chiang et al 1989; Irwin et al 1990; Liu et al 1992). In addition to regulating the ionization processes of the drugs, co-solvents may alter the barrier properties which in turn modify the transport profile. As a result, co-solvents used in the vehicle may exert a profound influence on the percutaneous delivery from topical dosage forms, thereby providing a way of engineering the desired penetration rate for topical products by adjusting the ratio of the co-solvents used.

Since the thermodynamic activity of the drug in the vehicle and the partition coefficient between the stratum corneum and the vehicle are very susceptible to the composition in the vehicle, any qualitative or quantitative variation in one or more of the components rapidly affects the penetrating rate of the drug through the skin (Ashton et al 1988). The importance of the solubility of the solutes in a binary solvent in determining the delivering rate into the skin has already been discussed (Berner et al 1989). The effects of the solubility characteristics of the penetrant, as well as the partitioning behaviour, on the relative rates of percutaneous absorption of two types of prostaglandin has been presented by Watkinson et al (1991). It was also demonstrated that the theoretical value of the partition coefficient, K_m, showed a good relationship with the experimentally determined permeability, K_p (Sloan et al 1986).

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Recently, Pardo et al (1990) reported the optimization of percutaneous delivery of physostigmine from a binary solvent based on the concept of thermodynamic control. A method to predict the skin permeability from theoretical partition coefficients determined by cohesion parameters was proposed by Matitani et al (1993). In this paper, the effect of co-solvents on the in-vitro penetration of indomethacin through the skin of hairless mice from a gel dosage form is examined.

Materials and Methods

Materials

Indomethacin was supplied by Medichem SA (Gerona, Spain). Di-isopropanolamine, glycerine, PEG 400, isopropanol, and propylene glycol were obtained from Merck Co., Cleveland, OH. Carbomer 940 was provided by BF Goodrich (Schuchart, Germany). Other reagents used were of analytical grade.

Preparation of gel dosage forms

Gel dosage forms of indomethacin were prepared using a serial mixture of de-ionized water, propylene glycol (or PEG 400) and ethanol (or isopropanol) 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 co-solvent mixture. After complete hydration of Carbomer by the vehicle, 1 g drug was added and mixed until homogeneous. The mixture was gelified by the dropwise addition of 50% di-isopropanolamine aqueous solution, and then the vehicle was made up to 100 g. The volume of neutralizing agent added resulted in a pH of approximately 5 for the gel products, at which indomethacin has maximal stability. The pH value of the gel products was determined in the solution by dissolving or dispersing 0·5 g gel in 50 g de-ionized water.

Solubility study

An excess of indomethacin 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 indomethacin was determined by UV-absorbance measurement.

In-vitro penetration study

Hairless mice (strain ICR), aged 6–10 weeks and weighing approximately 20 g, were obtained from the National Defense Medical Center, Taipei, Taiwan, ROC. The mice

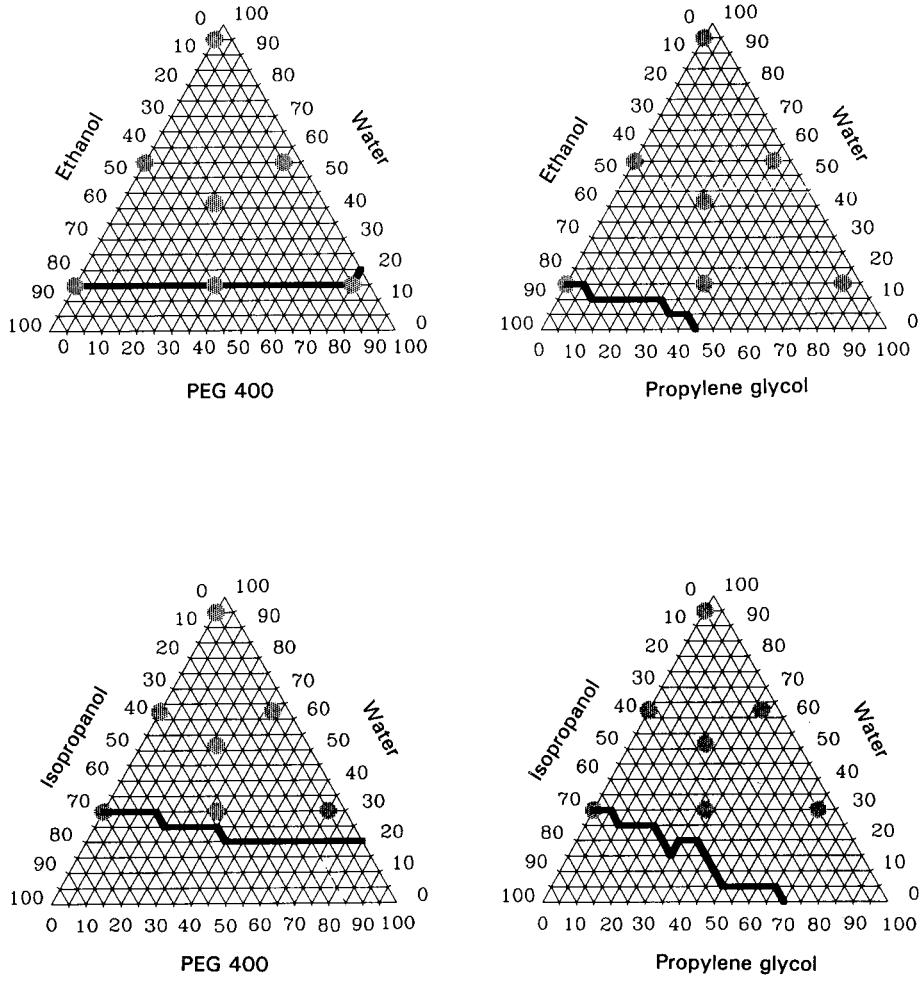

FIG. 1. A phase diagram for four three-component mixtures. The region above the solid line represents the vehicle mixtures producing a gel and the circles represent the formulations selected in the simplex lattice experimental design.

were killed by spinal dislocation. Fresh skin was excised from the abdominal region, and was washed with 0·9% NaCl (saline) before being placed on a Franz-type 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 phosphate buffer (pH 6·8, 50 mM) was used as the receptor medium and maintained at a temperature of 37°C, using a circulating water jacket, with stirring at a constant rate of 500 rev min^{-1}. The dermis of the skin was in contact with the receiver compartment. At predetermined time intervals, 200-μL aliquots were withdrawn from the receiver compartment and an equal volume of fresh medium was added to maintain a constant volume. The determination of indomethacin was carried out by an HPLC method.

Analytical procedure

HPLC analyses were undertaken using a system constructed from a Jasco 980 pump, a Jasco UV/VIS Detector-975, and a reversed-phase column (Lichrosphor RP-Select B 12·5 cm × 4·0 mm i.d., 5 μm). Samples were mixed with 200 μL internal standard solution containing mefenamic acid at a concentration of 20 $\mu g\,mL^{-1}$ and a volume of 20 μL was injected onto the HPLC system. Measurements were taken at a wavelength of 265 nm. The mobile phase consisted of methanol and 20 mM acetate buffer (pH = 5·0) at a volume ratio of 65 to 35, and a delivery rate of 1 mL min^{-1}. The retention time of indomethacin and mefenamic acid were about 3·5 and 5·5 min, respectively.

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 (or PEG 400) and ethanol (or isopropanol) in the vehicle. Seven formulations were studied for each co-solvent mixture in the respective triangular space, as shown in Fig. 1. These formulations included one at each vertex, one at the halfway point between the vertices, and one at the centre point. With the restriction that the sum of the total weights must be equal to 100 g, both the active ingredient and Carbomer 940 were kept at 1% w/w.

At steady state, the flux (J_{ss}) through the skin membrane is constant and is expressed by:

$$J_{ss} = DK_m\Delta C_s/h \cong DK_mC_d/h = K_m(h/(6t_L))C_d \quad (1)$$

where D is the diffusion coefficient in the stratum corneum, K_m is the partition coefficient between the stratum corneum and the gel body, h is the thickness of the stratum corneum, and $\Delta C_s/h$ is the concentration gradient across the stratum corneum. Since indomethacin has a good solubility in the receptor fluid at neutral pH, it was possible to maintain the sink condition throughout the study. Therefore, the concentration gradient ($\Delta C_s/h$) will be approximately equal to C_d/h, in which C_d is the drug concentration in the gel.

When the steady-state portion of the line is extrapolated to the time axis, the point of intersection is known as the lag time, t_L, and is given by $h^2/6 \cdot D$. As a result, D/h can be calculated from $h/(6t_L)$. By definition, K_m is expressed as the ratio of the solubility of the solute i in the stratum corneum (C_{sc}) to the solubility of the solute i in the gel (C_{gel}) so that

$$J_{ss} \cdot C_{gel} = ((D/h) \cdot C_{sc}) \cdot C_d \quad (2)$$

$$J_{ss} \cdot C_{gel} = ((h/(6t_L) \cdot C_{sc}) \cdot C_d \quad (3)$$

Based on equation 2, the enhancing effect of the co-solvent on the penetration of the drug can be separated from the effect of the partition coefficient by normalization with respect to the drug concentration (C_d) in the gel system (Dugard & Scott 1986). In the vehicle with a drug solubility greater than 10 mg mL^{-1}, C_d was set at 10 mg mL^{-1}, which is the maximal concentration of indomethacin which was added to the gel system. Otherwise, C_d was assumed to be the drug solubility in the corresponding vehicle. The enhancement factor (EF) of the co-solvent can then be calculated by normalization with respect to the value of $J_{ss}C_{gel}/C_d$ for the formulations A11 or I11 (Tables 1, 2). Based on equation 3, the effect of the co-solvent on the enhancement of drug solubility in the skin was evaluated by substituting D/h with $h/(6t_L)$, in which h is the mean

Table 1. The content of the formulations and their physical characteristics for water-alcohol-PEG 400 and water-alcohol-propylene glycol systems.

	A11	A21	A31	A41	A51	A61	A71	A42	A52	A62	A72
Formulations (% w/w)											
Ethanol	5 (0%)	45 (50%)	85 (100%)	45 (50%)	5 (0%)	5 (0%)	31·7 (33%)	45 (50%)	5 (0%)	5 (0%)	31·7 (33%)
PEG 400	0 (0%)	0 (0%)	0 (0%)	40 (50%)	80 (100%)	40 (50%)	36·7 (33%)				
Propylene glycol								40 (50%)	80 (100%)	40 (50%)	36·7 (33%)
Water	95 (100%)	55 (50%)	15 (0%)	15 (0%)	15 (0%)	55 (50%)	31·7 (34%)	15 (0%)	15 (0%)	55 (50%)	31·7 (34%)
Properties											
Solubility (mg mL^{-1})	0·043[a] (0·008)	1·48 (0·06)	23·01 (1·96)	53·29 (0·51)	53·51 (7·06)	0·73 (0·013)	5·04 (0·046)	14·15 (0·59)	6·64 (0·49)	0·27 (0·009)	1·76 (1·15)
Solubility parameter[b]	22·88	18·72	14·56	13·32	12·08	17·48	16·52	15·28	16·00	19·44	17·83
Flux (J_{ss} $\mu g\,cm^{-2}\,h^{-1}$)	1·34 (0·31)	4·81 (0·50)	12·73 (3·27)	3·98 (1·45)	2·85 (2·27)	2·35 (0·35)	7·13 (0·71)	15·82 (1·57)	3·81 (0·60)	1·58 (0·31)	4·24 (0·62)
Lag time (h)	3·3 (0·57)	3·47 (0·45)	6·98 (0·45)	7·12 (0·45)	8·02 (0·62)	3·1 (0·75)	1·64 (0·81)	4·03 (1·78)	3·12 (0·95)	5·98 (0·50)	1·21 (0·79)
Thickness/lag time (mm h^{-1})	0·121 (0·023)	0·133 (0·019)	0·070 (0·021)	0·063 (0·006)	0·042 (0·006)	0·129 (0·034)	0·321 (0·129)	0·080 (0·015)	0·117 (0·048)	0·091 (0·016)	0·333 (0·176)
EF $[J_{ss} \cdot C_{gel}/D/h)/C_d]$	1·0	3·27	37·63	30·64	32·76	1·64	2·01	25·31	2·93	1·58	1·15
EF $[J_{ss} \cdot C_{gel}/C_d]$	1·0	3·59	21·86	15·83	11·38	1·75	5·32	16·71	2·84	1·18	3·16

[a]Mean (s.d.). $\Sigma\phi_y\delta_y$, where ϕ_y is the volume fraction of constituent having solubility parameter δ_y.

Table 2. The content of the formulations and their physical characteristics for water-isopropanol-PEG 400 and water-isopropanol-propylene glycol systems.

	I11	I21	I31	I41	I51	I61	I71	I42	I52	I62	I72
Formulations (% w/w)											
Isopropanol	5 (0%)	37·5 (50%)	70 (100%)	37·5 (50%)	5 (0%)	35 (0%)	36·7 (33%)	37·5 (50%)	5 (0%)	5 (0%)	36·7 (33%)
PEG 400	0 (0%)	0·0 (0%)	0 (0%)	32·5 (50%)	65 (100%)	32·5 (50%)	31·7 (33%)				
Propylene glycol								32·5 (50%)	65 (100%)	32·5 (50%)	1·7 (33%)
Water	95 (100%)	62·5 (50%)	30 (0%)	30 (0%)	30 (0%)	2·5 (50%)	31·7 (34%)	30 (0%)	30 (0%)	32·5 (50%)	31·7 (34%)
Properties											
Solubility ($mg\,mL^{-1}$)	0·078[a] (0·003)	2·46 (0·02)	21·42 (1·31)	29·59 (2·31)	16·85 (1·53)	0·35 (0·05)	4·16 (0·33)	10·33 (0·13)	1·97 (0·15)	0·15 (0·004)	2·39 (0·07)
Solubility parameter[b]	22·81	18·94	15·07	14·55	14·03	18·42	17·32	16·14	17·22	19·37	18·38
Flux (J_{ss} $\mu g\,cm^{-2}\,h^{-1}$)	0·81 (0·10)	5·01 (0·51)	10·57 (2·31)	11·41 (2·93)	1·87 (0·19)	1·09 (0·37)	4·54 (0·18)	11·66 (2·56)	2·82 (0·33)	2·38 (0·50)	4·61 (0·52)
Lag time (h)	4·25 (1·27)	3·89 (1·34)	6·37 (0·28)	5·72 (0·71)	6·32 (0·56)	2·14 (1·43)	2·33 (0·82)	4·72 (1·21)	5·39 (0·35)	4·5 (0·33)	3·58 (1·27)
Thickness/lag time ($mm\,h^{-1}$)	0·137 (0·045)	0·172 (0·109)	0·090 (0·012)	0·074 (0·007)	0·073 (0·005)	0·336 (0·390)	0·198 (0·091)	0·093 (0·028)	0·076 (0·016)	0·080 (0·006)	0·154 (0·072)
EF ($J_{ss} \cdot C_{gel}/(D/h)/C_d$)	1·0	4·90	42·54	76·92	7·31	0·55	3·86	21·83	6·26	4·99	5·03
EF ($J_{ss} \cdot C_{gel}/C_d$)	1·0	6·19	27·95	41·68	3·89	1·35	5·60	14·87	3·48	2·94	5·69

[a]Mean (s.d.). [b]$\Sigma\phi_y\delta_y$, where ϕ_y is the volume fraction of constituent having solubility parameter δ_y.

thickness of the skin measured before and after the penetration study and t_L was obtained as described above. Similarly, the enhancement factor, $J_{ss}C_{gel}/(D/h)/C_d$, of the co-solvent on the drug solubility in the skin was calculated by normalization with respect to that of A11 or I11.

Results and Discussion

The transport behaviour of indomethacin across hairless mouse skin was investigated using a gel dosage form, prepared by gelling four different solvent mixtures of water, either propylene glycol or PEG 400 and either ethanol or isopropanol, with Carbomer 940. The boundary (the solid line) in the phase diagram of the ability to form a gel structure after neutralization with di-isopropanolamine was defined, and the results are shown in Fig. 1 (the region above the solid line). A simplex lattice design was employed to optimize the solvent ratio with the desired penetration rate and is represented by a triangle within this region, as shown in Fig. 1 (circles). Seven formulations were selected for each solvent mixture (Tables 1, 2). The solubility parameters of the co-solvent system were calculated as the sum of the products of the volume fraction and the solubility parameter for each solvent assuming there was no significant change in volume on mixing (Barton 1983). Drug solubility in the corresponding vehicle was measured.

In addition to the drug itself, the pH value of the vehicle and the composition of the co-solvent in the gel vehicle are two other important factors to consider in the evaluation of drug penetration from a gel dosage form across the membrane or the skin. The pH value affects the balance between the ionized and un-ionized forms of the drug. However, the ionized and un-ionized forms should show different penetration behaviour and rates as previously reported (Kushla & Zatz 1991; Hayashi et al 1992). On the other hand, the composition of the co-solvent in the gel body will affect the ionization constant (pK_a) and the solubility of the drug in the vehicle and also determine the solubility parameter of the vehicle, which in turn influences the partition coefficients of the drug between the gel body and the skin. An increase of co-solvent levels not only increases the pK_a value of the drug, but also increases the solubility of the un-ionized form of the drug. Increasing pK_a means the fraction of the un-ionized form of the drug increases if the pH value of the system is unchanged. However, the increasing solubility of the un-ionized form of the drug is only able to enhance the penetrating rate significantly when the dissolved amount of the drug increases when increasing the co-solvent levels. Otherwise, the increasing solubility of the un-ionized form of the drug results in a decrease of partition coefficient between the stratum corneum and the vehicle. Obviously, the composition of the co-solvent in the gel system has profound and complex effects on the flux. In addition, penetration of the co-solvent modifies the structure of the skin, thus altering the resistance to the diffusion path across the skin and also the solubility of the drug in the skin. Therefore, all formulations were purposely prepared with similar pH values (around 5) to equalize any pH effect or minimize the difference of pH effect. The effects of the composition of the co-solvent on the penetration rate of the drug across the skin were elucidated based on the difference in the solubility parameter and the solubility of the drug and their influence on the skin structure.

When using hairless mouse skin as the barrier, the penetration rate is obviously controlled by the skin membrane (Fig. 2). The penetration rate at steady state for each formulation was calculated based on equation 1, and the results are listed in Tables 1 and 2. Furthermore, this simplex design allows easy construction of a polynomial equation with seven terms that quantitatively fits the resulting data:

$$\text{Response} = b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 \quad (4)$$

where X_1, X_2, and X_3 represent the transformed percentages of the concentrations for water, alcohol (or isopropanol), and PEG 400 (or propylene glycol), respectively. Using penetration rate as the response variable, the coefficients

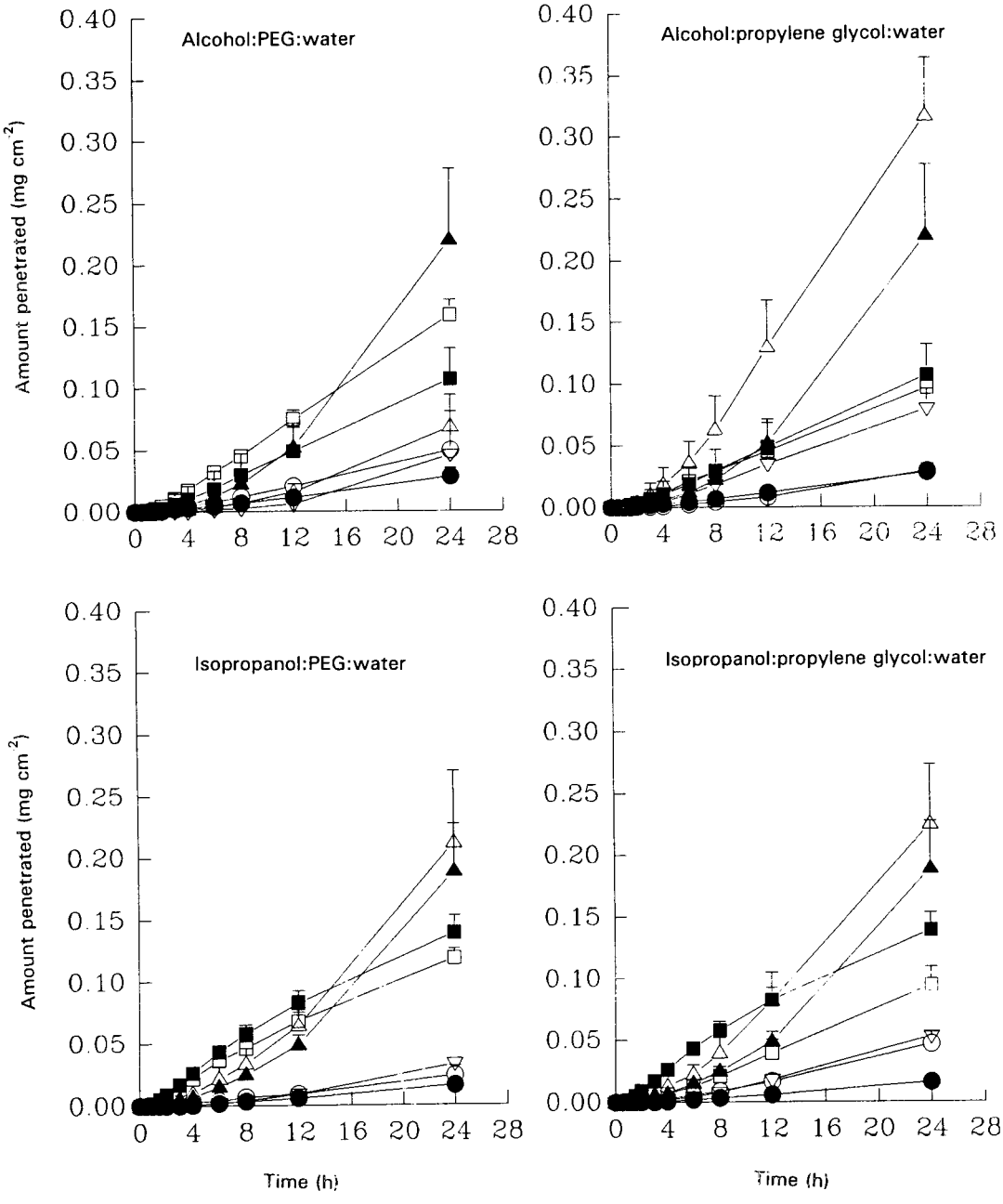

FIG. 2. The plots of the penetration rate vs time through hairless mouse skin. A11 and I11 (●); A21 and I21 (■); A31 and I31 (▲); A41, A42, I41 and I42 (△); A51, A52, I51, and I52 (▽); A61, A62, I61 and I62 (○); A71, A72, I71 and I72 (□).

in the following response equations are obtained (Bolton 1986) and should represent the response surface in the simplex space for each solvent mixture:

$$\text{Rate (ethanol-PEG)} = 1{\cdot}34X_1 + 12{\cdot}73X_2 + 2{\cdot}85X_3 - 8{\cdot}90X_1X_2 + 1{\cdot}02X_1X_3 - 15{\cdot}24X_2X_3 + 109{\cdot}59X_1X_2X_3 \quad (5)$$

$$\text{Rate (ethanol-propylene glycol)} = 1{\cdot}34X_1 + 12{\cdot}73X_2 + 3{\cdot}81X_3 - 8{\cdot}90X_1X_2 - 3{\cdot}98X_1X_3 + 30{\cdot}20X_2X_3 - 98{\cdot}40X_1X_2X_3 \quad (6)$$

$$\text{Rate (isopropanol-PEG)} = 0{\cdot}81X_1 + 10{\cdot}57X_2 + 1{\cdot}87X_3 - 2{\cdot}72X_1X_2 - 1{\cdot}00X_1X_3 + 20{\cdot}76X_2X_3 - 47{\cdot}79X_1X_2X_3 \quad (7)$$

$$\text{Rate (isopropanol-propylene glycol)} = 0.81X_1 + 10{\cdot}57X_2 + 2{\cdot}82X_3 - 2{\cdot}72X_1X_2 + 2{\cdot}26X_1X_3 + 19{\cdot}86X_2X_3 - 61{\cdot}53X_1X_2X_3 \quad (8)$$

Fig. 3 illustrates a three-dimensional plot of the penetration rate (z-axis) vs the percentage of water (x-axis) and alcohol (y-axis, or isopropanol) in the vehicle based on each empirical equation for the corresponding solvent mixtures.

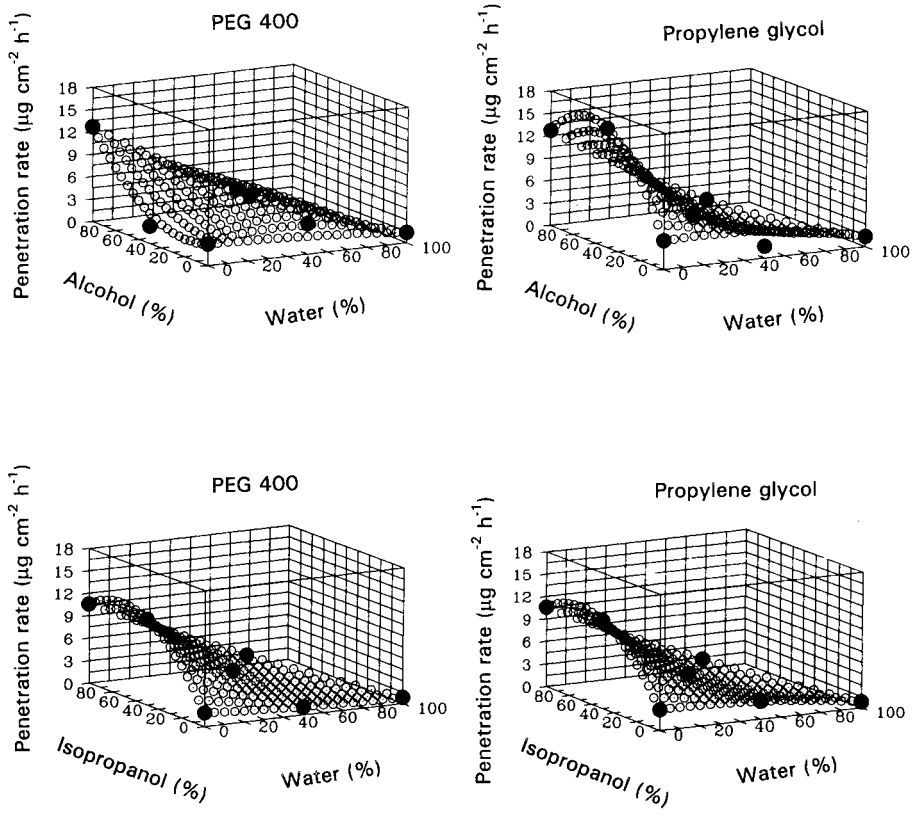

FIG. 3. A three-dimensional plot of the penetration rate through hairless mouse skin vs the vehicle composition based on the empirical equation obtained from the simplex lattice experimental design.

For a water-alcohol-PEG 400 mixture, a transformed alcohol concentration of 100% (formulation A31; the actual volume percentage of alcohol to water in the gel formulation was 85–15%), showed the highest penetration rate (12·74 $\mu g\,cm^{-1}\,h^{-1}$) in this simplex surface. For a water-alcohol-propylene glycol mixture, the formulation consisting of a transformed alcohol concentration of 65% and propylene glycol concentration of 35% (of which the actual volume ratio was water : alcohol : propylene glycol = 15 : 33 : 52), had the highest penetration rate (16·49 $\mu g\,cm^{-2}\,h^{-1}$) in this simplex surface. For both water-isopropanol-PEG 400 and water-isopropanol-propylene glycol solvent mixtures, the highest penetration rate (12·32 and 12·41 $\mu g\,cm^{-2}\,h^{-1}$, respectively) was observed in the formulation with a transformed percentage of isopropanol to PEG 400 (or propylene glycol) equal to 70/30, of which the actual volume ratio of water : isopropanol : PEG 400 (or propylene glycol) = 30/50·5/19·5.

An attempt to correlate the penetration rate through the skin and the drug solubility in the corresponding solvent mixture, with the solubility parameter of the vehicle was made. Fig. 4 shows that the plot of logarithmic solubility of the drug in the vehicle vs the solubility parameter of the vehicle gives a straight line with a very good correlation coefficient. This appears to demonstrate that the solubility of indomethacin in these polar co-solvents is better described by this relationship. According to Hildebrand's extended theory, the closer the solubility parameters of solute and solvent, the higher will be the solubility of the

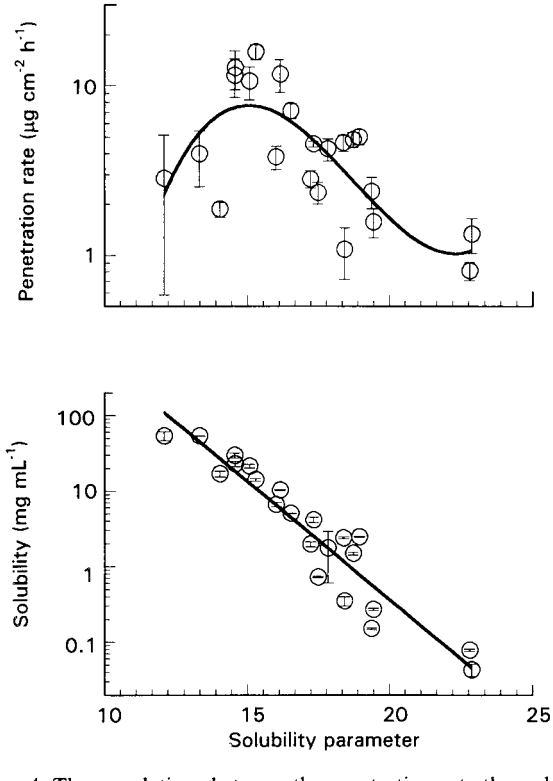

FIG. 4. The correlations between the penetration rate through the hairless mouse skin (above) or the drug solubility in the gel matrix (below) and the solubility parameter in the respective vehicle.

Table 3. Group contribution method for calculating molar volume and cohesion parameters of indomethacin.

Group	Number	Energy of evaporation ($cal\ mol^{-1}$)	Molar volume ($cm^3\ mol^{-1}$)
Indole	1	12660	70·2
Phenyl	1	7630	52·4
CO	1	4150	10·8
O	1	800	3·8
CH_3	2	1125 × 2	33·5 × 2
CH_2	1	1180	16·1
COOH	1	6600	28·5
Cl	1	2300	26·0
Sum		37570	274·8
σ			11·69

solute in the solvent. The solubility parameter of indomethacin was calculated to be 11·69 by James (1986) and is listed in Table 3. Thus A41 (13·32) and A51 (12·08) show the highest drug solubility for indomethacin.

However, the plot of the logarithmic penetration rate vs the solubility parameter shows a curve with the highest penetration rate at a solubility parameter of 15. The penetration rate decreases with a decreasing solubility parameter for those vehicles with a solubility parameter < 15. The penetration rate also decreases with an increasing solubility parameter when the solubility parameter of the vehicle > 15. This clearly indicates that the solubility in the vehicle is an important factor influencing the penetration of indomethacin into the skin. It was interesting to discover that the drug solubility in the vehicle having a solubility parameter of 15 was around $10\ mg\ mL^{-1}$, which is just about the maximal concentration of indomethacin that was added to the gel system. Thus, when the solubility parameter of the vehicle is equal to 15, the solubility in the vehicle is adequate to dissolve all the drug in the gel system, resulting in the maximum drug activity in the system. However, when the solubility parameter of the vehicle < 15, the solubility of the vehicle is unable to dissolve all the drug in the system, which thus results in a decrease in the drug activity. Furthermore, if the solubility parameter of the vehicle > 15, the solubility in the vehicle is not only able to make the added drug completely dissolve, but also increases the tendency for the drug to be retained in the gel system, which thus impairs the penetration rate. As a result, the highest penetration rate occurred at a solubility parameter of 15. In addition, from the relationship existing between the solubility parameter and the solubility, it appears to be possible to predict what kind of co-solvent composition will give the highest penetration rate for various amounts of indomethacin added in the solvent mixture.

According to Fick's law (also eqn 1), C_d, K_m and D/h are three important factors to consider concerning the penetration of drugs through the skin. C_d is determined by the concentration of the drug in the gel system, which is a function of the solubility parameter of the vehicle as discussed above. K_m is influenced by the drug solubility in both the vehicle and in the skin. The latter may be changed by contact with different solvent systems resulting from the penetration of the co-solvent. The diffusion coefficient of the drug in the skin is mainly dependent on the characteristics of the penetration path in the skin, which in turn is also dependent on the various modifications due to the co-solvent on the skin. As described above, the effect of the vehicle on the penetration rate can be explained completely by the effect on the solubility in the gel system through the solubility parameter. Furthermore, the modifications by the co-solvent on the skin which influence the partition coefficient and the diffusion coefficient in the skin should also be considered.

Overall, the influence of the co-solvent on the penetration of drugs can be considered as the simultaneous effect on the partition coefficient and the diffusion coefficient via the influence on the skin structure. Based on equation 2, the effect of the co-solvent system on skin structure, designated as the enhancement factor, $J_{ss}C_{gel}/C_d$, could possibly be separated from the effect on the drug solubility in the vehicle, and was calculated by normalization with respect to the $J_{ss}C_{gel}$ value of A11 or I11. In the water-alcohol-PEG and water-alcohol-propylene glycol solvent systems, a transformed alcohol concentration of 100% for alcohol resulted in the highest enhancement factor (A31: 21·86), whereas a transformed percentage of 50:50 for propylene glycol and water resulted in the lowest (A62: 1·18). In the water-isopropanol-PEG and water-isopropanol-propylene glycol solvent systems, a transformed percentage of 50:50 for isopropanol:PEG resulted in the highest enhancement factor (I41: 41·68), whereas a transformed percentage of 50:50 for propylene glycol and water resulted in the lowest (I62: 2·94). Using the enhancement factor as a response, similar empirical equations to those of the penetration rate were derived from the experimental design. The coefficients of the empirical equation for each solvent system are listed

Table 4. The coefficients of empirical equations derived from simplex experiments using enhancement factors as a response.

$J_{ss} \cdot C_{gel}(D/h)/C_d$	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_{123}
Alcohol-PEG	1·00	37·63	32·76	−64·17	−60·96	−18·22	−158·2
Alcohol-propylene glycol	1·00	37·63	2·93	−64·17	−1·56	20·10	−206·1
Isopropanol-PEG	1·00	42·54	7·31	−67·47	−14·44	207·97	−731·5
Isopropanol-propylene glycol	1·00	42·54	6·26	−67·47	5·43	−10·30	−95·23
$J_{ss} \cdot C_{gel}/C_d$	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_{123}
Alcohol-PEG	1·00	21·86	11·38	−31·36	−17·75	−3·17	−7·70
Alcohol-propylene glycol	1·00	21·86	2·84	−31·36	−2·97	17·42	−95·15
Isopropanol-PEG	1·00	27·95	3·89	−33·16	−4·40	103·04	−340·7
Isopropanol-propylene glycol	1·00	27·95	3·48	−33·16	2·79	−3·39	−36·96

in Table 4. The coefficients may be regarded as indicators of the extent of change in the relative enhancement factor for each co-solvent. As shown in Table 4, the coefficient of b_2 indicates that the extent of change in the enhancement factor on the skin for both alcohol and isopropanol is most profound, with a 20-fold increase over that of water. There was only a threefold increase over that of water for propylene glycol and PEG 400, except for that of PEG 400 in a water-alcohol-PEG solvent system which had an 11-fold increase.

According to equation 2, the enhancement factor ($J_{ss} \cdot C_{gel}/C_d$) is a function of the diffusion coefficient, D, the thickness of the stratum corneum, h, and the drug solubility in the stratum corneum, C_{sc}, assuming that the drug concentration in the gel, C_d, did not change significantly during the steady state. The penetration of the co-solvent into the stratum corneum is necessary for the co-solvent to exert its effects on the skin structure. Simultaneous alterations by the co-solvents of these three parameters are possible. Therefore, the overall effect of the co-solvent on $J_{ss} \cdot C_{gel}/C_d$ should be determined by the individual effect on each parameter. By substituting D/h with $h/6(t_L)$, it is possible to separate further the effect of the co-solvent on the diffusion path in the skin from that on the drug solubility in the skin. Similarly, the enhancement factor ($J_{ss} \cdot C_{gel}/(D/h)/C_d$) with respect to the drug solubility in the skin was evaluated. As indicated in Tables 1 and 2, the effect of the co-solvent on the drug solubility in the skin showed a similar tendency to that of the enhancement factor. Using ($J_{ss} \cdot C_{gel}/C_d$) the enhancement factor ($J_{ss} \cdot C_{gel}/(D/h)/C_d$) as a response, similar empirical equations to that for the penetration rate were also derived from the experimental design. The coefficients of the empirical equation for each solvent system are listed in Table 4. The extent of change in the relative enhancement factor for these co-solvents on drug solubility was similar to that on the enhancement factor ($J_{ss} \cdot C_{gel}/C_d$).

Conclusions

The highest penetration rate occurred with the co-solvent mixture of water : alcohol : propylene glycol = 15 : 33 : 52, which also possessed a drug solubility which was adequate to dissolve all the drug added to the system. A prediction of the optimal penetration rate for various concentrations of indomethacin from the co-solvent systems examined, appears to be possible by utilizing the solubility parameter of the co-solvent. On the other hand, the concentration of indomethacin in the vehicle is the main factor in controlling the penetration rate despite the fact that different co-solvents enhance penetration rate to different extents. This may be due to the closeness of the solubility parameter of indomethacin to that of skin ($\sigma = 10$), resulting in the fact that the concentration of indomethacin becomes a determinant factor. It would be valuable to further explore how the difference in the solubility parameters between the solute and the skin affects the mechanisms controlling the penetration of solute into the skin.

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