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Enhanced transdermal delivery of estradiol in vitro using binary vehicles of isopropyl myristate and short-chain alkanols

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Abstract

The effect of binary vehicles of isopropyl myristate (IPM) and short-chain alkanols on the enhancement of skin permeation of estradiol (E_2) was studied in vitro using human epidermal membrane. The steady-state fluxes of E_2 and solvents across the skin were determined from saturated solutions of neat and binary solvents of IPM and ethanol (EtOH), *n*-propanol (*n*-PrOH), *n*-octanol (*n*-OcOH), or isopropanol (i-PrOH). While the neat solvents modestly increased the E_2 flux, addition of IPM to the alkanols resulted in a synergistic enhancement of the E_2 flux. Among the (1:1) binary cosolvents evaluated, i-PrOH produced the highest E_2 flux (1.1 $\mu g/cm^2$ per h), which was 35-fold greater than from water and over 15-fold greater than from the neat solvents. This combination was also the best in terms of relative compositions of the IPM/i-PrOH cosolvents. A strong correlation between E_2 and i-PrOH fluxes suggested the enhancement for both permeants. While i-PrOH traversed the skin, IPM was retained in the stratum corneum. The uptake of both IPM and E_2 in the stratum corneum was largely increased by adding i-PrOH (up to 50%) to IPM.

Keywords: Transdermal delivery, in vitro; Binary enhancer; Isopropyl myristate; Short-chain alkanol; Human skin; Estradiol

1. Introduction

Human stratum corneum with its intercellular lipid multilayers is generally accepted as the rate-limiting barrier in transdermal drug delivery (Blank et al., 1967; Behl et al., 1982). The use of penetration enhancing solvents is valuable and important for achieving therapeutic plasma levels of many drugs (Loth, 1991; Stantus and Baker, 1993). These enhancers can be generally classified as polar (hydrophilic) or non-polar (hydrophobic). For example, the polar enhancer ethanol (EtOH) has been employed in commercially successful estradiol (E_2) transdermal systems (Good et al, 1986). Other short-chain alkanols have also been reported as enhancers in hairless mouse

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skin (Ghanem et al., 1987; Kai et al., 1990; Kim et al., 1992; Krill et al., 1992) and in human skin (Friend et al., 1988; Kurihara-Bergstrom and Liu, 1991; Liu et al., 1991). With its established use in many pharmaceutical and cosmetic preparations, isopropyl myristate (IPM), a non-polar enhancer, is well tolerated topically (Holzner, 1963; Campbell and Bruce, 1981). Binary enhancers of these two classes may offer synergistic enhancement and reduced skin irritation (Cooper et al., 1985; Catz and Friend, 1990; Pardo et al., 1990).

The purpose of this study was to determine the effect of binary enhancers of IPM and short-chain alkanols on the transport of E_2, a lipophilic model compound across human epidermal membrane in vitro. The alkanols used in this study included the primary alkanols, EtOH, *n*-propanol (*n*-PrOH), and *n*-octanol (*n*-OcOH), and a secondary alkanol, isopropanol (i-PrOH). E_2 solubilities and transdermal deliveries of E_2 and solvents were determined using neat and IPM/alkanol binary solvents. With IPM/i-PrOH mixtures, stratum corneum-to-solution partition coefficients of E_2, IPM, and i-PrOH were evaluated in comparison with their fluxes across the skin.

In the skin transport experiments, saturated E_2 solutions of the various solvents were used to maintain an equal and unit thermodynamic activity of E_2. The steady-state E_2 fluxes were normalized to that from water, as an indicator of the enhancement factor (EF), defined as:

$$EF = J_{E_2,\text{enhancer}}/J_{E_2,\text{water}} \quad (1)$$

where $J_{E_2,\text{enhancer}}$ and $J_{E_2,\text{water}}$ are the fluxes from the tested solutions and from water, respectively. The EF accounts for the increase of E_2 solubility and/or diffusivity in the stratum corneum (Liu et al., 1991).

2. Materials and methods

2.1. Chemicals and human skin

E_2 (Diosynth, The Netherlands), absolute EtOH (US Industrial Chemical Co., Tuscola, IL), IPM and *n*-OcOH (Aldrich Chemical Co., Milwaukee, WI), and certified *n*-PrOH and HPLC-grade i-PrOH (Fisher Scientific, Fair Lawn, NJ) were used as received. Aqueous solutions were prepared with deionized distilled water. Gentamicin (Sigma, St. Louis, MO) was used as an antibiotic in the skin experiments.

Human split-thickness cadaver skin, dermatomed from the back region, was obtained from a skin bank. The skin samples had been cascade frozen and stored at −70°C until used. For the in vitro skin permeation experiment, epidermal membrane (stratum corneum + viable epidermis) was isolated from the dermis by immersion in saline solution containing 0.01% gentamicin at 60°C for 2 min (Kligman and Christophers, 1963). For the partition experiment, stratum corneum was obtained by skin digestion (Kligman and Christophers, 1963) in saline solution containing 0.1% trypsin and 0.01% gentamicin, at room temperature for 24 h. The separated stratum corneum sheet was dried over night under vacuum at room temperature and stored desiccated until use.

2.2. Analytical methods

As previously described, E_2 was assayed by reverse-phase HPLC (Liu et al., 1991) and EtOH by GC (Kurihara-Bergstrom et al., 1990). *n*-PrOH and i-PrOH were also analyzed using this GC method. At a carrier gas flow rate of 12 ml/min, the retention times of EtOH, i-PrOH, and *n*-PrOH were 1.9, 2.8, and 3.9 min, respectively.

For the GC assay of *n*-OcOH, a 10 foot × 2 mm i.d. glass column packed with 10% silicone OV-101 on Chromosorb WHP 80/100 mesh (Alltech Associates, Inc., Deerfield, IL) was used. The column temperature was ramped from 142 to 190°C at 12°C/min and the injection port and detector temperatures were 210 and 265°C, respectively. The retention time was 3.3 min using a flow rate of 12 ml/min. The GC method for IPM utilized the same instrument and carrier gas as the *n*-OcOH method described above, except the glass column was 3 m × 2 mm i.d. and the temperatures for column, injector port, and detector were 170, 205, and 250°C, respectively. The retention time for IPM was 4.8 min with a flow rate of 10 ml/min.

2.3. Solubility experiment

E_2 solubilities were determined for various neat and binary solvents. An excess amount of E_2 was added to the tubes containing the vehicles. The tubes were stirred for 72 h at 32°C and then centrifuged for 30 min at 32°C, 3400 rpm. E_2 concentrations in the supernatant solutions were analyzed by HPLC. All solutions had reached equilibrium saturation within 72 h.

2.4. Determination of IPM / alkanol / water phase diagrams

IPM/alkanol/water phase diagrams were determined in the absence of E_2. By fixing the weight ratios of two components (IPM and alkanols), the weight percentage of the third component (water) at which phase separation occurred was determined by visual inspection. The point at which the solution became cloudy was an indicator of phase separation. A small weight increment was made with a 250 μl syringe and measured by an electronic balance.

IPM and *n*-OcOH were miscible at any combinations, but neither of them was practically soluble in water. The other alkanols were miscible with both IPM and water over the entire range and had similar phase diagrams as shown in Fig. 1A (for IPM/i-PrOH/water system). Fig. 1B presents IPM/*n*-OcOH/water system. These phase diagrams were useful in the in vitro skin permeation experiment designed to prevent unwanted phase separation in the drug reservoir due to the depletion of water-miscible solvent components and/or the back diffusion of water into the reservoir.

2.5. Determination of stratum corneum-to-solution partition coefficients

The volume-based apparent stratum corneum-to-solution partition coefficients of E_2, IPM, and i-PrOH were simultaneously determined at 32°C in the various IPM/i-PrOH binary solutions saturated with E_2. Each stratum corneum sample (10 μm × 1 cm^2) was incubated in 5 ml of the bulk solution and shaken at 100 rpm. For all solutions, equilibrium was reached within 72 h with no detectable changes of E_2, i-PrOH, and IPM concentrations in the bulk solution. After briefly wiping the surfaces dry with a Kimwipe, the thickness of stratum corneum was measured with an electronic micrometer (Mitutoyo Elecont, Japan). The stratum corneum was then extracted in ethanol (for E_2 and IPM) and in water (for i-PrOH) for a total of three times, each with 5 ml of the fresh solvents for 24 h. It was shown that > 99% of available amount for each compound was extracted using this procedure. The extracts were analyzed for E_2, IPM, and i-PrOH.

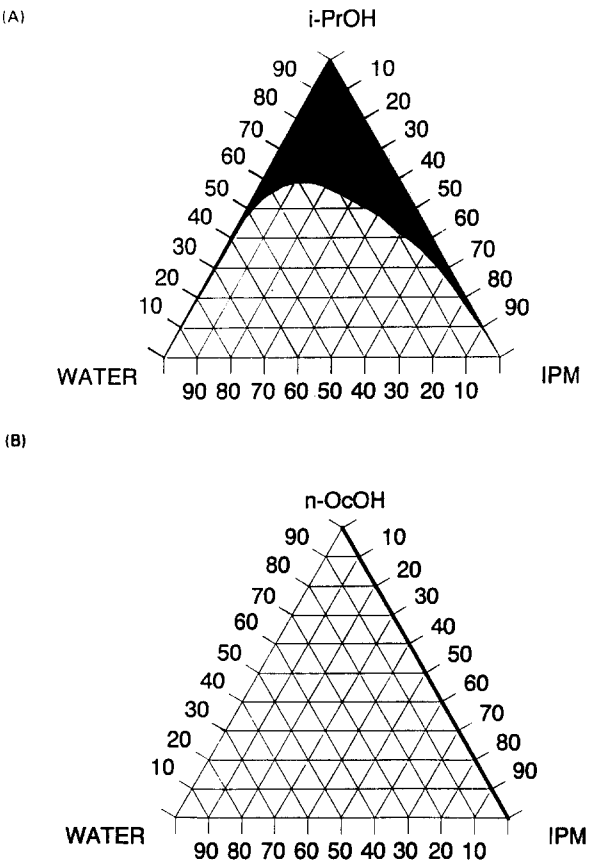

Fig. 1. IPM/alkanol/water phase diagrams (wt%) at room temperature (25 ± 1°C). The shaded area represents the miscible region. (A) IPM/i-PrOH/water system. (B) IPM/*n*-OcOH/water system.

2.6. In vitro skin permeation experiment

In vitro skin permeation experiments were performed at 32°C using two-chamber vertical flow-through diffusion cells (Laboratory Glass Apparatus Inc., Berkeley, CA). Human epidermal membrane was mounted on the diffusion cells with stratum corneum facing the donor chamber. The donor chamber was charged with 4 ml/3.3 cm^2 of E_2-saturated solution and then replaced frequently with fresh solution to ensure no significant changes in the vehicle composition due to phase separation (Fig. 1). The receiver sample effluent (saline containing 0.01% gentamicin) was automatically collected every 2 h for 48 h at the rate of approx. 3.5 ml/h so that sink conditions were always maintained. The actual collected volume was determined gravimetrically (i.e., taking density of 1 g/ml) and then analyzed for E_2, IPM, and alkanols. The steady-state fluxes were calculated from:

$$J = \frac{\left(\frac{dA_R}{dt}\right)}{S} = \frac{C_R Q_R}{S} \quad (2)$$

where S is the diffusional area, A_R and C_R denote the amount and concentration in the receiver chamber, respectively, Q_R is the receiver solution flow rate, and t represents time. The apparent permeability coefficients are calculated as the steady-state fluxes divided by the donor concentrations.

3. Results and discussion

3.1. Effect of neat solvents: IPM and alkanols

As a baseline study, the solubilities and skin fluxes of E_2 were determined in neat solvents of IPM and four short-chain alkanols. Table 1 summarizes these results along with the information for water. The E_2 solubilities in IPM and alkanols were 570- and more than 10 000-times greater than that in water, respectively. E_2 solubility in i-PrOH was 2-fold greater than in *n*-PrOH.

As shown in Table 1, *n*-OcOH enhanced E_2 flux 5-fold over that from water, while IPM, EtOH, *n*-PrOH, and i-PrOH only doubled the average flux (with no statistic significance for the two propanols). The effect of alkanol chain length on E_2 flux showed similar results to those of nicotinamide across hairless mouse skin (Kai et al., 1990). For the solvents' fluxes, EtOH was about 10-times higher than that for the two propanols, and neither IPM nor *n*-OcOH were detected in the receiver solution. E_2 fluxes from EtOH, *n*-PrOH and i-PrOH were the same while the solvent fluxes decreased with increasing the chain length. Calculated from the fluxes and

Table 1
Effect of neat solvents: solubility, steady-state fluxes, enhancement factor (EF) and apparent permeability coefficients at 32°C [a]

Neat solvent	E_2 solubility (mg/ml)	Steady-state flux (μg/cm^2 per h)		Average EF for E_2	Apparent permeability coefficient (cm/s)	
		E_2	Solvent		E_2	Solvent
H_2O	0.003 ± 0.001	0.031 ± 0.008	–	1.0	$(2.87 \pm 0.74) \times 10^{-6}$	–
IPM	1.7 ± 0.3	0.070 ± 0.012 [c]	– [b]	2.3	$(1.14 \pm 0.20) \times 10^{-8}$	– [b]
EtOH	31.0 ± 0.5	0.058 ± 0.015 [c]	803 ± 97	1.9	$(5.20 \pm 1.34) \times 10^{-10}$	$(2.83 \pm 0.34) \times 10^{-7}$
n-PrOH	42.1 ± 0.9	0.055 ± 0.036 [d]	75 ± 2	1.8	$(3.63 \pm 2.37) \times 10^{-10}$	$(2.59 \pm 0.07) \times 10^{-8}$
n-OcOH	42.0 ± 0.2	0.189 ± 0.053 [c]	– [b]	6.1	$(1.25 \pm 0.35) \times 10^{-9}$	– [b]
i-PrOH	95.4 ± 0.2	0.059 ± 0.037 [d]	92 ± 10	1.9	$(1.72 \pm 1.08) \times 10^{-10}$	$(3.26 \pm 0.35) \times 10^{-8}$

[a] Solubilities ($n = 3$) and steady-state fluxes ($n = 3$–5 from two skin donors) were determined at 32°C and presented as average ± standard deviation. EF (enhancement factor) was calculated as defined by Eq. 1. Apparent permeability coefficients were the steady-state fluxes divided by the donor concentration. Densities of IPM, EtOH, *n*-PrOH, *n*-OcOH, and i-PrOH are 0.853, 0.789, 0.805, 0.827, and 0.785 g/ml, respectively, at 20°C (Merck Index, 11th Edn, 1989).
[b] IPM and *n*-OcOH fluxes were under the GC detection limits due to their immiscibility with water.
[c] Significantly different ($p < 0.05$) in single-tailed *t*-test (compared to water).
[d] Not significantly different ($p \geq 0.05$) in single-tailed *t*-test (compared to water).

donor concentrations, the apparent permeability coefficients of E_2 and solvents are also presented in Table 1. With different solvents, the E_2 permeability coefficient was in the order of $H_2O \gg$ IPM > *n*-OcOH > EtOH ≥ *n*-PrOH ≥ -PrOH. This is mainly because of the E_2 stratum corneum-to-solvent partition coefficients, which decrease in the same order. The neat alkanols' permeability coefficients were essentially in parallel to their flux data.

A modest enhancement of E_2 flux by neat alkanols may be explained by a combination of two opposing factors. Reduction of the diffusional barrier by extracting stratum corneum lipids and proteins increases with increasing chain length of alkanols, which is found sufficiently extensive with *n*-OcOH (Kai et al., 1990; Goates at al., 1994). On the other hand, dehydration of the stratum corneum makes it less permeable (Blank et al., 1984). The mechanism of action of neat IPM is poorly understood, however, despite IPM's well-established skin tolerability and wide applications in pharmaceuticals and perfumery.

3.2. Effect of alkanols on the IPM / alkanol (1:1) binary cosolvents

The effects of IPM/alkanol (1:1) binary cosolvents with different alkanols were compared to the neat solvents. Table 2 presents the E_2 solubility, fluxes and apparent permeability coefficients of E_2 and alkanols, and EF values for E_2. When IPM and alkanols were mixed at equal weight, the E_2 solubilities were mainly dominated by the alkanols.

Both E_2 and alkanol fluxes were substantially increased using the IPM/alkanols binary cosolvents. The E_2 fluxes from the cosolvents of three primary alkanols were similar, in the range of 0.27–0.49 $\mu g/cm^2$ per h with EF = 9–16. The highest E_2 flux of approx. 1.1 $\mu g/cm^2$ per h was obtained from the cosolvent of IPM and the branched i-PrOH. This was a 35-fold enhancement compared to water and more than 15-fold enhancement compared to either neat IPM or neat i-PrOH. A synergistic enhancement effect is clearly demonstrated by the binary cosolvent systems. Interestingly, while the effectiveness of the two neat propanols was the same (Table 1), the i-PrOH/IPM cosolvent was a more potent enhancer (by a factor of 4) than the *n*-PrOH/IPM cosolvent. Compared to the neat alkanol, the E_2 fluxes were enhanced approx. 2-, 5-, 8- and 18-fold by adding IPM to *n*-OcOH, *n*-PrOH, EtOH and i-PrOH, respectively. Being in the same order (10^{-9} cm/s) for all the IPM/alkanol (1:1) binary cosolvents, the E_2 permeability coefficients were increased (over the neat alkanols) by approximately the same magnitude as the E_2 fluxes. This may be attributed to the enhancement of E_2 diffusivity in the stratum corneum by adding IPM in the alkanols.

Regarding the alkanol transport from the IPM/alkanol (1:1) binary cosolvents, both the

Table 2
Effect of IPM/alkanols (1:1) binary cosolvents: solubility, steady-state fluxes, enhancement factor (EF), and apparent permeability coefficients at 32°C [a]

Binary cosolvent (equal weight)	E_2 solubility (mg/ml)	Steady-state flux ($\mu g/cm^2$ per h)		Average EF for E_2	Apparent permeability coefficient (cm/s)	
		E_2	Alkanol		E_2	Alkanol
IPM/EtOH	30.8 ± 0.3	0.49 ± 0.24 [c]	4011 ± 1192	16	$(4.42 \pm 2.16) \times 10^{-9}$	$(2.71 \pm 0.81) \times 10^{-6}$
IPM/*n*-PrOH	38.1 ± 0.5	0.27 ± 0.11 [c]	399 ± 85	9	$(1.97 \pm 0.80) \times 10^{-9}$	$(2.68 \pm 0.57) \times 10^{-7}$
IPM/*n*-OcOH	29.3 ± 0.8	0.30 ± 0.05 [c]	– [b]	10	$(2.84 \pm 0.47) \times 10^{-9}$	– [b]
IPM/i-PrOH	68.7 ± 3.4	1.07 ± 0.21 [c]	2521 ± 429	35	$(4.33 \pm 0.85) \times 10^{-9}$	$(1.71 \pm 0.29) \times 10^{-6}$

[a] Solubilities ($n = 3$) and steady-state fluxes ($n = 3$–5 from two skin donors) were determined at 32°C and presented as average ± standard deviation. EF (enhancement factor) was calculated as defined by Eq. 1. Apparent permeability coefficients were the steady-state fluxes divided by the donor concentrations.
[b] *n*-OcOH flux was under the GC detection limit due to its immiscibility with water.
[c] Significantly different ($p < 0.05$) in single-tailed *t*-test (compared to water, Table 1).

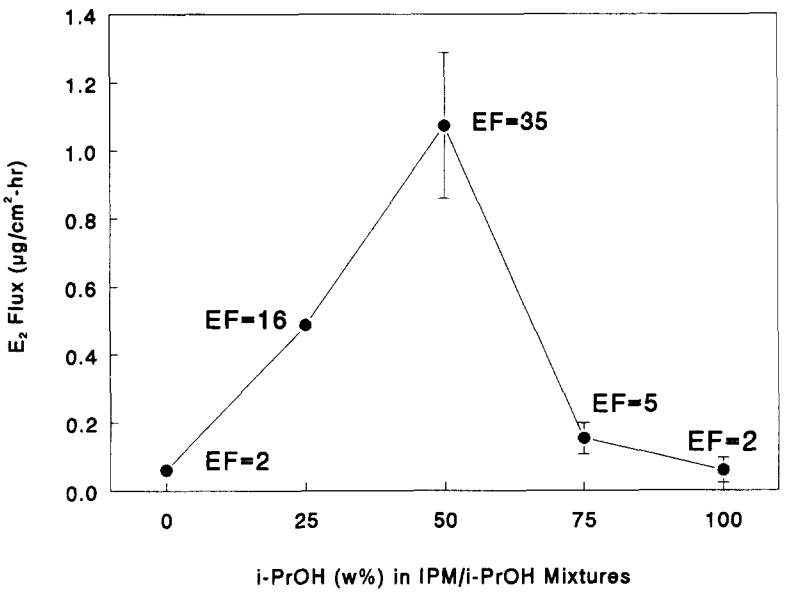

Fig. 2. E_2 steady-state fluxes through human epidermal membrane in vitro from the saturated E_2 donor solutions of IPM/i-PrOH mixtures and the average EF values for E_2 at 32°C ($n = 3$–5 from two skin donors).

EtOH and *n*-PrOH fluxes were increased 5-fold from the corresponding neat solvents. A 28-fold increase in i-PrOH flux was found with the IPM/i-PrOH cosolvent. As the alkanols were diluted by half in the equal weight binary cosolvents, the magnitude of increase in the alkanols' permeability coefficient was approximately twice as large as in the alkanols' flux. In contrast to the equal and unit thermodynamic activity of saturated E_2 in the all binary cosolvents, different alkanols in the equal weight IPM/alkanol cosolvents may not have the same partial vapor pressures. This is one of the reasons why the fluxes of E_2 and alkanols are not correlated as shown in

Table 3
Effect of relative composition of IPM/i-PrOH binary cosolvents: solubility, apparent stratum corneum-to-bulk solution partition coefficients, and apparent permeability coefficients at 32°C [a]

Bulk solution		Stratum corneum-to-bulk solution Apparent partition coefficient			Apparent permeability coefficient (cm/s)	
i-PrOH/IPM (wt%)	Saturated E_2 (mg/ml)	E_2	i-PrOH	IPM	E_2	i-PrOH
0:100	1.7 ± 0.3	3.9 ± 2.8	–	0.9 ± 0.3	$(1.14 \pm 0.20) \times 10^{-8}$	–
25:75	33.8 ± 0.2	2.6 ± 0.9 [c]	0.05 ± 0.03 [c]	1.5 ± 0.3 [b]	$(4.03 \pm 1.37) \times 10^{-9}$	$(5.05 \pm 2.02) \times 10^{-7}$
50:50	68.7 ± 3.4	2.6 ± 1.0 [c]	0.06 ± 0.04 [c]	2.2 ± 1.0 [b]	$(4.33 \pm 0.85) \times 10^{-9}$	$(1.71 \pm 0.29) \times 10^{-6}$
75:25	86.6 ± 4.0	2.5 ± 0.9 [c]	0.04 ± 0.02 [c]	2.2 ± 0.6 [b]	$(1.57 \pm 0.07) \times 10^{-9}$	$(5.40 \pm 0.77) \times 10^{-7}$
100:0	95.4 ± 0.2	2.6 ± 1.5 [c]	0.09 ± 0.05	–	$(1.72 \pm 1.08) \times 10^{-10}$	$(3.26 \pm 0.35) \times 10^{-8}$

[a] E_2 solubilities or saturated E_2 ($n = 4$). The volume-based partition coefficient. The stratum corneum-vs-solution volume ratios were < 0.001 so that at equilibrium (< 72 h), the concentrations of E_2, i-PrOH, and IPM in the bulk solutions remained unchanged ($n = 6$–8 stratum corneum samples from two skin donors). Apparent permeability coefficients were the steady-state fluxes (Fig. 2 and 3) divided by the donor concentrations.

[b] Significantly different ($p < 0.05$) in single-tailed *t*-test (compared to neat IPM).

[c] Not significantly different ($p \geq 0.05$) in single-tailed *t*-test (compared to neat IPM for E_2 or to neat i-PrOH for i-PrOH).

Table 2. Another reason for this may be due to the structure-dependent enhancement for the different alkanols. However, the binary-to-neat flux ratio was nearly proportional for E_2 and for each alkanol, indicating the enhancement for both permeants.

3.3. Effect of relative composition in the IPM / i-PrOH binary cosolvents

The IPM/i-PrOH cosolvents were selected to examine the effect of binary composition, since the IPM/i-PrOH (1:1) mixture demonstrated the highest E_2 flux enhancement (Table 2). Fig. 2 presents the E_2 flux as a function of relative composition of the IPM/i-PrOH binary cosolvents. The flux of E_2 across the skin was highest (35-fold enhancement) from an equal weight of i-PrOH in IPM. The binary cosolvents containing 25 and 75% i-PrOH also enhanced E_2 flux 16- and 5-fold, respectively. This composition-dependent enhancement has been reported for levonorgestrel with ethyl acetate/EtOH binary cosolvents (Catz and Friend, 1990). With increasing i-PrOH concentration in the cosolvents, the apparent permeability coefficient of E_2 decreased, by approx. 100-fold from neat IPM to neat i-PrOH (Table 3). Again, this is generally consistent with the decrease in the E_2 partition coefficients.

The fluxes of co-transported i-PrOH are presented in Fig. 3, showing a very similar pattern to the E_2 fluxes (Fig. 2). A linear correlation was observed when plotting E_2 flux vs i-PrOH flux for the i-PrOH concentrations of 25%-100% (w/w) in IPM. Such apparent linear dependence of the solvent flux was also observed for nitroglycerin in EtOH/water mixtures (Berner et al., 1989) and E_2 in EtOH/water mixtures (Liu et al., 1991). In spite of changing i-PrOH thermodynamic activity with its concentration in the binary cosolvents, this flux correlation may indicate that the lipoidal pathway dominates the transport of both E_2 and i-PrOH across the stratum corneum and this pathway is enhanced for both E_2 and i-PrOH by the IPM/i-PrOH cosolvents. Similar to the flux data, the apparent permeability coefficient of i-PrOH was maximized around the equal weight mixture of IPM and i-PrOH (Table 3).

Table 3 also presents E_2 solubilities and apparent stratum corneum-to-solution partition coefficients in the IPM/i-PrOH mixtures. As expected, the E_2 solubility increased with increas-

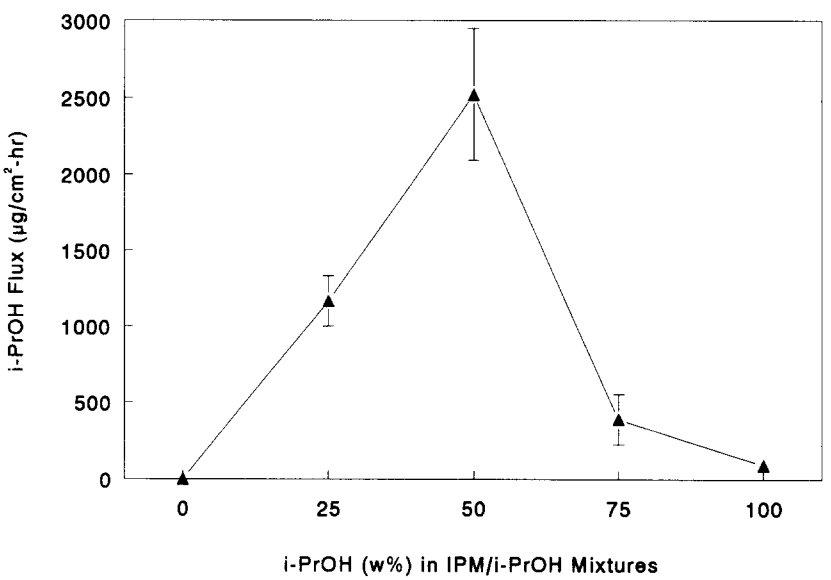

Fig. 3. i-PrOH steady-state fluxes through human epidermal membrane in vitro from the same experiments as in Fig. 2. IPM flux was under the GC detection limit due to its immiscibility with water.

ing i-PrOH in the mixtures. The E_2 partition coefficients were determined to be 2–3 and independent of the IPM/i-PrOH compositions. In parallel to the E_2 solubility in the mixtures, the E_2 uptake (solubility) in the stratum corneum sharply increased (about 27-fold) from neat IPM to 50 wt% i-PrOH. From 50 wt% i-PrOH to neat i-PrOH, the increase was more gradual (37%). That is, the change of E_2 diffusivity in stratum corneum could be a dominating action of the mixtures with 50 wt% or higher i-PrOH. Complicated by the i-PrOH gradient across the stratum corneum, the E_2 uptake profile was not directly comparable with the E_2 flux data (Fig. 2).

As also summarized in Table 3, IPM and i-PrOH showed much different properties of partitioning between the stratum corneum and the IPM/i-PrOH mixtures. The partition coefficient for neat IPM was 0.9 ± 0.3, which was 10-times that for neat i-PrOH. For a wide range of IPM/i-PrOH compositions, the i-PrOH partition coefficient was unchanged, but addition of i-PrOH to IPM significantly increased the IPM partition coefficient. Clearly, the polar i-PrOH traversed the skin and the non-polar IPM was largely retained in the stratum corneum; both aspects make the i-PrOH/IPM combination a unique enhancer system. The stratum corneum retention of IPM is consistent to the recent FT-IR studies showing that the non-polar enhancer oleic acid exists as a liquid within the stratum corneum lipids, which may cause interfacial defects within the lipid bilayers (Ongpipattanakul et al., 1991).

4. Conclusions

The binary cosolvents of IPM and short-chain alkanols (EtOH, *n*-PrOH, *n*-OcOH, and i-PrOH) significantly enhanced the transport of E_2 through human skin in vitro compared to the single solvents. Among the (1:1) binary cosolvents evaluated, the branched alkanol, i-PrOH, produced the highest E_2 flux (1.1 $\mu g/cm^2$ per h), which was 35-fold greater than from water and more than 15-fold greater than from the individual neat solvents. This enhancement was also dependent upon the cosolvent compositions of IPM/i-PrOH mixtures. E_2 solubility in the stratum corneum increased with increasing i-PrOH in the mixtures. This combination of i-PrOH diffusion across and IPM retention in the stratum corneum offers synergistic enhancement in transdermal delivery for other lipophilic drugs. Depending on the relative compositions of the cosolvent, this synergistic enhancement may be due to a combination of increasing both diffusivity and solubility of E_2 in the stratum corneum. Additional experiments are required to further understand the enhancement mechanism(s) of this novel enhancer system.

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References

Behl, C.R., Barrett, M., Flynn, G.L., Kurihara, T., Walters, K.A., Gatmaitan, O.G., Harper, N, Higuchi, W.I., Ho, N.F.H. and Pierson, C.L., Hydration and percutaneous absorption: III. Influence of stripping and scalding on hydration alteration of the permeability of hairless mouse skin to water and *n*-alkanols. *J. Pharm. Sci.*, 71 (1982) 229–234.

Berner, B., Mazzenga, G.C., Otte, J.H., Steffens, R.J., Juang, R.H. and Ebert, C.D., Ethanol:water mutually enhanced transdermal therapeutic system: II. Skin permeation of ethanol and nitroglycerin. *J. Pharm. Sci.*, 78 (1989) 402–407.

Blank, I.H., Maloney, J., Emslie, A.G. and Simon, I., The diffusion of water across the stratum corneum as a function of its water content. *J. Invest. Dermatol.*, 82 (1984) 188–195.

Blank, I.H., Scheuplein, R.J. and MacFarlane, D.J., Mechanism of percutaneous absorption: III. The effect of temperature on the transport of non-electrolytes across the skin. *J. Invest. Dermatol.*, 49 (1967) 582–589.

Campbell, R.L. and Bruce, R.D., Comparative dermatotoxicology: I. Direct comparison of rabbit and human primary skin irritation responses to isopropylmyristate. *Toxicol. Pharmacol.*, 59 (1981) 555–563.

Catz, P. and Friend, D.R., Effect of cosolvents on ethylacetate enhanced percutaneous absorption of levonorgestrel. *J. Controlled Release*, 12 (1990) 171–180.

Cooper, E.R., Merritt, E.W. and Smith, R.L., Effect of fatty

acids and alcohols on the penetration of acyclovir across human skin in vitro. *J. Pharm. Sci.*, 74 (1985) 688–689.

Friend, D., Catz, P., Heller, J., Reid, J. and Baker, R., Transdermal delivery of levonorgestrel: I. Alkanols as permeation enhancers in vitro. *J. Controlled Release*, 7 (1988) 243–250.

Ghanem, A.H., Mahmoud, H., Higuchi, W.I., Rohr, U.D., Borsadia, S., Liu, P., Fox, J.L. and Good, W.R., The effects of ethanol on the transport of lipophilic and polar permeants across hairless mouse skin: II. A new quantitative approach. *J. Controlled Release*, 6 (1987) 75–83.

Goates, C.Y. and Knutson, K., Enhanced permeation of polar compounds through human epidermis: I. Permeability and membrane structural changes in the presence of short-chain alcohols. *Biochim. Biophys. Acta*, (1994) in press.

Holzner, C., The use of isopropyl fatty esters in cosmetics formulations. *Am. Perfum. Cosmet.*, 78 (1963) 89–93.

Kai, T., Mak, V.H.W., Potts, R.O. and Guy, R.H., Mechanism of percutaneous penetration enhancement: effect of *n*-alkanols on the permeability barrier of hairless mouse skin. *J. Controlled Release*, 12 (1990) 103–112.

Kim, Y., Ghanem, A., Mahmoud, H. and Higuchi, W.I., Short chain alkanols as transport enhancers for lipophilic and polar/ionic permeants in hairless mouse skin: mechanism(s) of action. *Int. J. Pharm.*, 80 (1992) 17–31.

Kligman, A.M. and Christophers, Preparation of isolated sheets of human stratum corneum. *Arch. Dermatol.*, 88 (1963) 702–705.

Krill, S.L., Knutson, K. and Higuchi, W.I., Ethanol effects on the stratum corneum lipid phase behavior. *Biochim. Biophys. Acta*, 1112 (1992) 273–280.

Kurihara-Bergstrom, T. and Liu, P., Enhanced in vitro skin transport of ionized terbutaline using its sulfate salt form in aqueous isopropanol. *STP Pharm. Sci.*, 1 (1991) 52–59.

Kurihara-Bergstrom, T., Knutson, K., DeNoble, L.J. and Goates, C.A., Percutaneous absorption enhancement of an ionic molecule by ethanol-water systems in human skin. *Pharm. Res.*, 7 (1990) 762–766.

Liu, P., Kurihara-Bergstrom T. and Good W.R., Cotransport of estradiol and ethanol through human skin in vitro: understanding the permeant/enhancer flux relationship. *Pharm. Res.*, 8 (1991) 938–944.

Loth, H., Vehicular influence on transdermal drug penetration. *Int. J. Pharm.*, 68 (1991) 1–10.

Ongpipattanakul, B., Burnette, R.R., Potts, R.O. and Francoeur, M.L., Evidence that oleic acid exists in a separate phase within stratum corneum lipids. *Pharm. Res.*, 8 (1991) 350–354.

Pardo, A., Shiri, Y. and Cohen, S., Percutaneous absorption of physostigmine: optimization of delivery from a binary solvent by thermodynamic control. *J. Pharm. Sci.*, 79 (1990) 573–578.

Santus, G.C. and Baker, R.W., Transdermal enhancer patent literature. *J. Controlled Release*, 25 (1993) 1–20.