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RESEARCH PAPER

The Effect of Water on a New Binary Transdermal Flux Enhancer (Peg_3-Me/IPP): An In Vitro Evaluation Using Estradiol

E. Hansen,[1] J. Sclafani,[2,*] P. Liu,[1] and J. Nightingale[3]

[1]*CIBA, Suffern, New York 10901*
[2]*Guilford Pharmaceuticals, Baltimore, Maryland 21224*
[3]*Bend Research, Bend, Oregon 97701-8599*

ABSTRACT

Isopropyl palmitate (IPP), a skin penetration enhancer, combined with triethylene glycol monomethyl ether (Peg_3-Me) results in an excellent transdermal flux enhancer. A solution of 11% Peg_3-Me/IPP saturated with estradiol delivers the drug at a 60-fold greater rate than from estradiol (E_2)-saturated donors of IPP or Peg_3-Me alone. Unfortunately, a steady-state flux is not maintained. Studies using vertical permeation cells indicated that the back flux of water causes the donor solution to phase separate, with an IPP-rich phase floating away from the skin. The ternary phase diagram for IPP, Peg_3-Me, and H_2O shows that a solution of IPP/Peg_3-Me will only accept 1% water before phase separating. Additional experiments, involving donor solution replacement and reorientation of the skin relative to the donor solution, demonstrated that phase separation was responsible for the non-steady-state E_2 flux. Finally, a prototype bilayer laminate, which included a hydrophobic polyisobutylene layer (PIB), minimized the water flux from the receiver chamber into the donor and produced a sustained and high transdermal flux. While the mechanism of enhancement is complex, the Peg_3-Me/IPP flux enhancers may provide significant improvements for transdermal drug delivery.

*To whom correspondence should be addressed.

INTRODUCTION

A key hurdle in the development of transdermal drug delivery systems is achieving a drug flux that is high enough for therapeutic applications without damaging the skin. To overcome this, considerable effort has been applied to identifying flux enhancers. Selection of a flux enhancer is based on the permeation of the therapeutic agent and the enhancer, as well as available and acceptable local and systemic toxicological profiles. A stable formulation that is amenable to system design is also an important consideration (1).

A new binary enhancer was investigated which results in a dramatic increase in the flux of E_2 (estradiol). This enhancer is a combination of Peg_3-Me (triethylene glycol monomethyl ether) and IPP (isopropyl palmitate). Because of the phase relationship of the enhancer components, the back flux of water has an effect on the physical stability of the donor solution. This instability was examined through a series of permeation experiments using E_2 as a model compound. First, the donor solution was periodically replaced. Second, the skin was reoriented relative to the donor solution. Finally, a hydrophobic PIB (polyisobutylene) laminate was placed between the skin and the donor solution.

Substantial toxicological data are available for both components of the enhancer. IPP is commonly used in topical products and does not readily penetrate the skin (2). Peg_3-Me is used in many industrial and commercial products including brake fluids, paints, and cleaners; therefore, EPA-mandated testing has been performed in several animal models (3–5).

MATERIALS AND METHODS

Estradiol was received from Ciba-Geigy (Suffern, NY). IPP was obtained from Adhesives Research, Inc. (Glen Rock, PA). Peg_3-Me was obtained from Aldrich Chemical Company (Milwaukee, WI). Gentamicin sulfate was purchased from Sigma Chemical Co. (St. Louis, MO). Polyisobutylene (Vistanex) was received from Exxon Chemical Company while National Starch and Chemical Company (Bridgewater, NJ) provided a self-cross-linking acrylic adhesive: Duro-Tak 80-1194. ScotchPak 1022 and Cotran microporous membrane 9710 were obtained from 3M (St. Paul, MN). Materials for high-performance liquid chromatography (HPLC) analysis were of HPLC grade. All other chemicals were reagent grade.

Assay Method

Estradiol was quantified by HPLC using a 3-cm Perkin-Elmer ODS column with a mobile phase of 40/60 acetonitrile/water flowing at 1.5 ml/min. UV detection at 200 nm gave a retention time of 3 minutes.

IPP was quantified by gas chromatography (GC) on a 2-m 3% Carbowax 20M 80/100 mesh Supecloport column using nitrogen as the carrier gas at a flow rate of 10 ml/min. Temperatures of the FID detector, injector, and oven were 250°, 200°, and 200°C, respectively. This gave a retention time of 4.7 min.

Peg_3-Me was quantified by using the same GC configuration as for IPP with an oven temperature of 220°C. The retention time was 4 min. No IPP was detected in receiver solution samples.

Solubility

The E_2 solubilities were determined by adding excess E_2 to the binary solvents. Samples were capped and stirred for a minimum of 24 hr at room temperature, protected from the light. Then, samples were centrifuged and the supernatant was analyzed by HPLC.

Phase Diagrams

Phase diagrams for IPP/Peg_3, Me/H_2O were determined in the absence of E_2. The weight ratios of two components were fixed and a third was added until phase separation occurred. Phase separation was determined by visual inspection and the results were plotted on the periphery of a tertiary phase diagram. Information regarding the phase behavior at compositions at the inner portion of the diagram was not determined.

Skin Permeation

The in vitro skin permeation was determined at 32°C using either 0.7-cm^2 side-by-side cells (Crown Glass, NJ) or 3.3-cm^2 LGA glass diffusion cells (Laboratory Glass Apparatus, CA). Both have stirred receiver chambers with a receiver solution flow of 6 ml/hr. Samples were collected in tared test tubes every hour. The receiver solutions contained 0.9% NaCl with 0.01% gentamicin sulfate. Human epidermis was isolated following the method of Kligman et al. (6).

The epidermis was placed on the cell and clamped in place with the stratum corneum side facing the donor

solution. In experiments in which the hydrophobic/adhesive laminate was used, the epidermis was spread carefully onto a watch glass, stratum corneum side up, and gently blotted dry using a tissue. The system was adhered to the skin and the skin/system was clamped on a LGA cell.

The 11% Peg_3-Me/IPP (w/w) donor solution consisted of IPP saturated with both E_2 and Peg_3-Me. It was prepared by adding an excess of E_2 to a solution of 25% Peg_3-Me/IPP (v/v). This solution phase separated due to the small amount of water present in the E_2 (4.26%). After saturation, the upper phase (11%Peg_3-Me/IPP, as per GC analysis) was applied to the skin.

Hydrophobic/adhesive laminates were solvent-cast, hand-drawn films. The acrylic adhesive was cast as supplied with a 20 mil gap-width knife onto a release liner. This was air dried for a half hour followed by a half hour in a 50°C convection oven. Then, a microporous membrane was gently adhered to the adhesive. The PIB, 1.2×10^6 dalton (MW), was prepared with IPP in a 1:2 ratio (PIB:IPP) in hexane (15% w/w solids). This was cast over the dried acrylic with a 30 mil gap-width knife and dried as above. A microporous membrane was placed on top of the PIB as well. The acrylic side of the laminate was adhered to the stratum corneum side of the skin before mounting on the diffusion cell. The donor solution was applied on top of the second microporous membrane.

RESULTS AND DISCUSSION

Solubility

The solubility of E_2 in Peg_3-Me/IPP and Peg_3-Me/H_2O is shown in Table 1. The solubility increased dramatically as the Peg_3-Me content increased. The very high solubility of E_2 in Peg_3-Me is likely an important factor in the permeation-enhancing properties of this binary system.

Skin Permeation

The in vitro permeation results from E_2-saturated donors of IPP, Peg_3-Me, and 11% Peg_3-Me/IPP are presented in Table 2. IPP or Peg_3-Me alone produced a low, steady-state delivery of E_2. As seen in Fig. 1, the combination of 11% Peg_3-Me/IPP gave a non-steady-state flux of E_2 that peaked at a 60-fold greater value than either solution alone. At the end of the experiment, the donors, which were initially one phase, had separated into two liquid phases: the lower phase was a Peg_3-Me/water-rich phase with E_2 crystals present on the surface of the skin; the supernatant was an IPP-rich phase. The E_2 flux from the two-phase donor was still 10-fold higher than that from the individual components at the end of the experiment. While the Peg_3-Me flux profile mirrors that of E_2, IPP permeation was not detected because of the extremely low aqueous solubility.

Phase Diagrams

The phase diagram for the Peg_3-Me in the presence of IPP and water is illustrated in Fig. 2. The shaded regions are areas of miscibility and define the boundary where two separate phases first appear. Peg_3-Me/IPP/H_2O has an unusual phase diagram in that the Peg_3-Me/IPP axis has two regions of miscibility separated by an

Table 1

Solubility

Percentage of Peg_3-Me in Solution (v/v)	E_2 Solubility in Peg_3-Me/IPP Solution (mg/ml)	E_2 Solubility in Peg_3-Me/Water Solution (mg/ml)
0	1.1	0.001
20	9.7	0.019
40	ND[a]	0.19
60	ND	2.2
80	ND	18.7
90	115	33.7
95	126	105
100	155	155

[a]ND: not determined due to phase separation.

Table 2

Permeation

IPP Donor	Peg_3-Me Donor		11% Peg_3-Me/IPP Donor	
E_2 Flux ($\mu g/cm^2/hr$)	E_2 Flux ($\mu g/cm^2/hr$)	Peg_3-Me Flux ($\mu g/cm^2/hr$)	E_2 Flux ($\mu g/cm^2/hr$)	Peg_3-Me Flux ($\mu g/cm^2/hr$)
0.05 ± 0.03	0.034	126.3 ± 8.4	2.9 ± 1.3	1389 ± 154

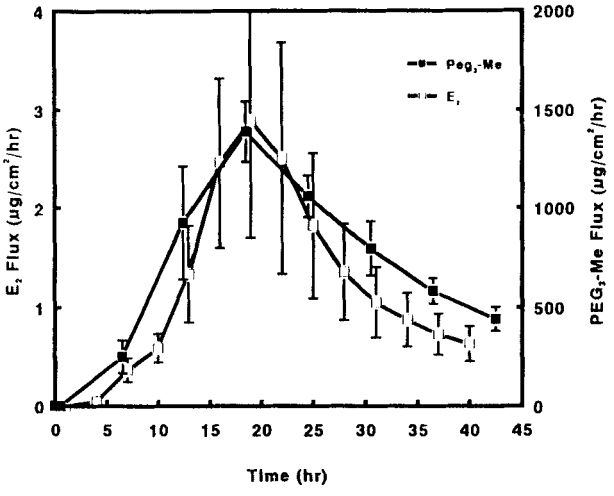

Figure 1. E_2 flux with 11% Peg_3-Me/IPP.

immiscible center region. Miscibility appears to be important for the permeation enhancement from this solvent system. The maximum amount of water in the miscibile regions is about 1% (w/w).

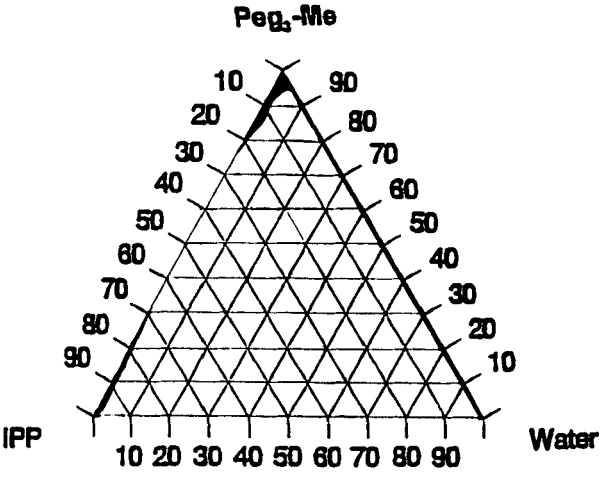

Figure 2. Phase diagram for Peg_3-Me in presence of IPP and water.

Examination of the Effect of the Back Flux of Water

Three approaches were taken to determine the effect of water entering the donor chamber on E_2 transport: (i) replacement of the donor solutions with fresh solution; (ii) changing the skin orientation so the skin remains in contact with the IPP phase; (iii) construction and use of a hydrophobic laminate limiting the back flux of water into the donor chamber.

The first experiment investigated the non-steady-state flux of E_2 by the replacement of the donor solution on horizontally mounted skin. During a 48-hr experiment, the donor solution was replaced at hours 3, 19, 27, and 43, identified in Fig. 3 as vertical lines. The replacement removed any accumulated water and restored the donor solution to the origional composition. After each replacement, the fluxes of both E_2 and Peg_3-Me increased. Note that where the fluxes were decreasing, the donor replacement caused a more significant increase in the flux. Donor separation had probably occurred and IPP was no longer in contact with the skin. Donor re-

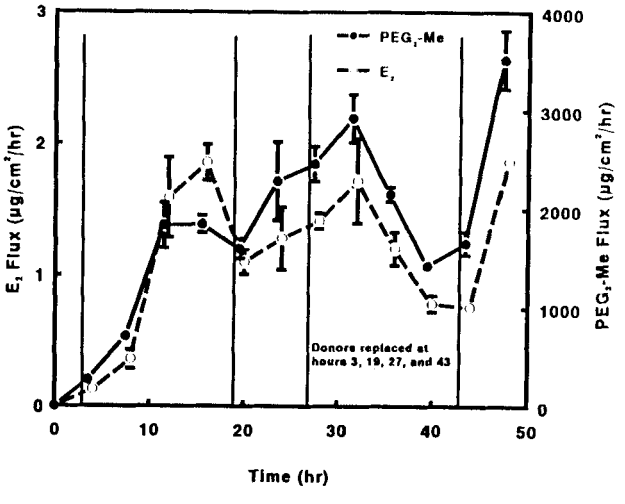

Figure 3. E_2 flux: replacement of donor solution.

placement restored IPP at the skin surface, resulting in increases in both fluxes.

In the second experiment, the skin orientation was changed from horizontal to vertical relative to the donor solution using side-by-side diffusion cells. In this configuration, when the solution phase separated into two phases, the IPP-rich phase remained in contact with the skin while the Peg_3-Me/water-rich phase accumulated below the diffusional area. As illustrated in Fig. 4, the fluxes of both E_2 and Peg_3-Me maintained high and constant levels comparable to the peak flux from the LGA cells (Fig. 1).

Finally, a bilayer laminate was constructed from PIB and an acrylic adhesive. The PIB hydrophobic layer limited the back flux of water, while the acrylic layer provided good skin adhesion. The donor solution did not phase separate. A seen in Fig. 5, the E_2 and Peg_3-Me fluxes remained high and constant throughout the 48-hr experiment. It is interesting to note that both fluxes are lower than those obtained without the laminate.

E_2 flux dependence on Peg_3-Me flux is illustrated in Fig. 6, where flux data from the laminate and skin orientation flux studies have been plotted. This figure shows an approximate linear relationship despite inclusion of non-steady-state values. The E_2/Peg_3-Me flux ratio is likely to depend on the skin donor. The observed linearity suggests cotransport of E_2 and Peg_3-Me, and a method of controlling E_2 delivery. Cotransport of E_2 and ethanol has been reported (7).

The complex mechanism of enhancement from this binary enhancer system is not elucidated in this study. Three results stand out: (i) the strong E_2 solubility increase with increasing Peg_3-Me concentration; (ii) the requirement that both enhancer components, Peg_3-Me and IPP, be present; and (iii) the apparent linearity of the E_2 flux with the Peg_3-Me flux. The conclusions of Liu et al. (7) regarding the mechanism of cotransport of E_2 and ethanol may apply to this system and serve as a starting point for further investigation. The mechanism may lie in combined effects of each component on the diffusivity and/or partition coefficient.

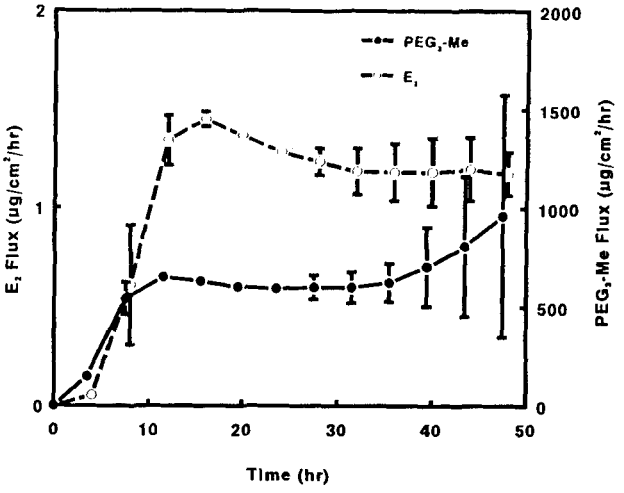

Figure 5. E_2 flux: use of binary laminate.

CONCLUSION

The binary flux enhancer of 11% Peg_3-Me/IPP showed an extraordinary 60-fold enhancement of the transdermal delivery of E_2. A series of carefully controlled experiments point to the back flux of water from

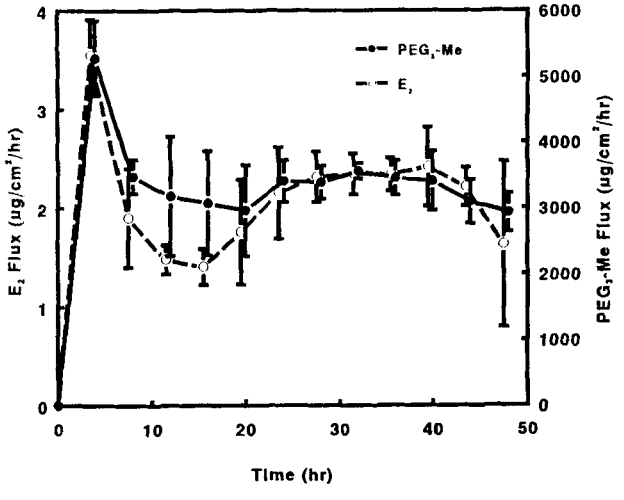

Figure 4. E_2 flux: changed skin orientation.

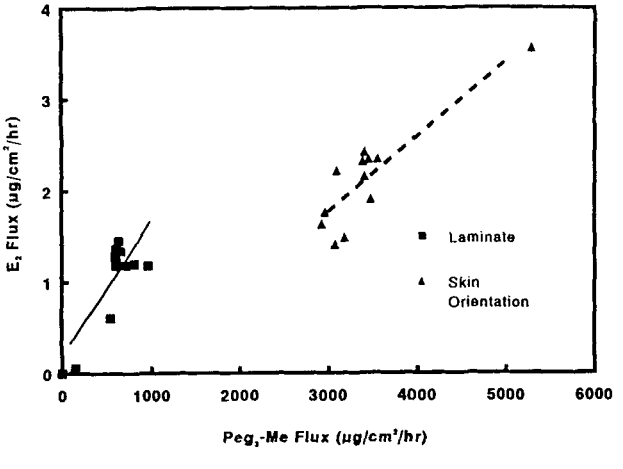

Figure 6. E_2 flux dependence on Peg_3-Me flux.

the receiver chamber into the donor chamber as causing the donor solution to separate into two phases. This separation appears to cause the observed non-steady-state flux of E_2. Investigation of the mechanism for Peg_3-Me/IPP flux enhancement requires further study. It is apparent that both components are necessary to obtain the full enhancement, and it appears that the delivery of E_2 can be controlled through the flux of Peg_3-Me.

A non-steady-state flux profile may be useful for pulsed or temporal therapies that require high but short-lived fluxes (i.e., hormones, cardiovascular medications) (8). A method was demonstrated to produce a constant delivery of E_2. The water back flux was reduced with a laminate and a steady-state flux of E_2 and Peg_3-Me was maintained for 2 days.

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