ELSEVIER

International Journal of Pharmaceutics 116 (1995) 101–112

international journal of pharmaceutics

Oestradiol permeation across human skin, silastic and snake skin membranes: the effects of ethanol/water co-solvent systems ☆

Nagia A. Megrab, Adrian C. Williams, Brian W. Barry *

Postgraduate Studies in Pharmaceutical Technology, The School of Pharmacy, University of Bradford, Bradford BD7 1DP, UK

Received 26 July 1994; modified version received 26 September 1994; accepted 30 September 1994

Abstract

Considerable interest in using the transdermal route for drug administration has strengthened the need for investigations of vehicle effects. The influence of ethanol on the in vitro transport behaviour of saturated oestradiol (OE) solutions through excised human skin and model membranes (silastic, human skin-silastic sandwich and snake skin) was investigated over 0–90% w/w ethanol/water vehicle compositions. Human skin showed a maximum flux of OE (1.45 ± 0.39 $\mu g\ cm^{-2}\ h^{-1}$) at ethanol vehicle contents between 40 and 60% w/w. Silastic membranes were used to help elucidate the mechanisms of ethanol as an enhancer. Partition coefficients and uptake of OE by stratum corneum and silastic membranes from the co-solvent systems were determined and the results suggested that the enhanced permeation of OE from vehicles with ethanol concentrations up to 60% w/w was partially related to increased drug solubility in the stratum corneum. The other part was related to ethanol effects on stratum corneum components. The decrease of OE flux from vehicles with higher ethanol concentrations was due to ethanol dehydration effects on the stratum corneum. This was confirmed by measuring the uptake of ethanol and water from different concentrations of ethanol; increasing ethanol concentration in the donor produced a significant decrease in the stratum corneum water content. Also, when the skin hydration was controlled using the skin-silastic sandwich model, the OE flux did not significantly decrease at high ethanol concentration. Ethanol actions were further investigated by measuring the permeation rate of OE through a model animal membrane, shed snake skin. Different results were obtained than for human skin and silastic membrane. The maximum flux of OE through dorsal snake skin appeared at higher ethanol vehicle concentration (80% w/w) when compared with human skin. Ventral snake skin showed increasing OE flux up to 40% w/w ethanol which remained essentially constant up to 90% ethanol. The results obtained with snake skin were attributed to the lower water content of this membrane.

Keywords: Estradiol; Ethanol/water; Human skin; Snake skin; Silastic membrane; Partition coefficient; Uptake

1. Introduction

Human skin consists of two distinct layers, the stratified avascular epidermis and an underlying dermis of connective tissue. The stratum corneum,

☆ Preliminary data were presented at the British Pharmaceutical Conference, Reading, UK, September 1993.

* Corresponding author. Postgraduate Studies in Pharmaceutical Technology, The School of Pharmacy, University of Bradford, Bradford BD7 1DP, UK.

SSDI 0378-5173(94)00321-1

which is the outermost layer of mammalian epidermis and consists of corneocytes embedded in a lipid matrix, typically provides the major barrier to most transdermal drug absorption (Blank, 1965; Barry, 1983). In recent years, growing interest in employing the transdermal pathway for administration of drugs for which a persistent systemic or local effect is desirable has emphasised the need for studies of vehicle effects on percutaneous absorption. Different vehicles have been identified as skin permeation enhancers such as propylene glycol and ethylene glycol (Mollgaard and Hoelgaard, 1983). Ethanol is a co-solvent used in topical pharmaceutical products and has been observed to increase permeation of a wide range of drugs through human and animal skins either in vivo or in vitro (Ghanem et al., 1987; Yum et al., 1987; Berner et al., 1989a; Pershing et al., 1990; Hatanaka et al., 1993; Obata et al., 1993). It is also used in at least three transdermal drug delivery systems: oestradiol (Campbell and Chandrasekaran, 1983), nitroglycerin (Gale and Berggen, 1986) and fentanyl (Gale et al., 1986).

Although there are numerous reports of ethanol effects on transdermal drug delivery, the mechanism of ethanol in promoting skin permeation is not completely clear. Some researchers have demonstrated an ethanol concentration-dependent enhancement mechanism, in which ethanol altered the lipid component of stratum corneum at low concentrations while at higher concentrations new pores formed in the stratum corneum (Ghanem et al., 1987). It has been suggested that the enhancement of skin permeability of a drug by ethanol at low concentration arises from physical perturbation of the lipoidal barrier region in the stratum corneum, while that at higher concentrations arises from conformation changes within the keratinised protein component and partial extraction of stratum corneum lipid (Knutson et al., 1990; Kurihara-Bergstrom et al., 1990). Additionally, a linear dependence of nitroglycerin skin permeation on the ethanol flux for ethanol volume fractions ≤ 0.7, resulting from the two permeants following each other through the skin, was reported (Berner et al., 1989a).

In the present study, the effects of various ethanolic solutions (0–90% w/w) on the permeation of β-oestradiol across human skin and model membranes (silastic, skin-silastic sandwich and snake skin) were investigated with the aim of elucidating the mechanisms of ethanol in promoting skin permeability. To avoid the complication arising from co-transport of water and ethanol through the membranes, donor and receptor solutions were always maintained at the same ethanol/water composition.

2. Materials and methods

2.1. Materials

The lipophilic permeant was β-[2,4,6,7-H^3(N)]oestradiol (NEN Dupont Research Products, Dreiech, Germany), radiochemical purity 99%. Unlabelled β-oestradiol (Sigma Chemical Co. Ltd, Poole, UK) was used to prepare saturated drug solutions. A medical grade non-reinforced silastic sheeting (0.005 inch thick) and a medical adhesive silicone type A were supplied by Dow Corning Corp. (Michigan, USA). Tritiated water was obtained from Amersham International plc (Bucks, UK), and [1-^{14}C]ethanol from Sigma Chemical Co. Ltd.

2.2. Solubility studies

Excess oestradiol was added to a series of ethanol/water co-solvent systems from 0 to 100% w/w. The suspensions were stirred using magnetic bar stirrers for 3 days in a water bath at 32 ± 1°C, then filtered through a PTFE membrane filter (pore size 0.2 μm). The concentrations of the saturated solutions were determined spectrophotometrically at λ_{max} (282 nm) after appropriate dilution with ethanol.

2.3. Diffusion studies

2.3.1. Preparation of the membranes

2.3.1.1. Human epidermal membranes. Caucasian human mid-line abdominal skin was obtained post mortem and stored in double sealed and evacuated polythene bags at −20°C until required

(Harrison et al., 1984). Epidermal membranes were prepared by a heat-separation technique (Kligman and Christophers, 1963). Excess fatty and connective tissues were removed from the skin which was then immersed in a water bath at 60°C for 45 s. The epidermis was teased away from the underlying dermis and floated on an aqueous solution of 0.002% w/v sodium azide for 72 h to ensure essentially full hydration of the stratum corneum. Skin samples from 17 donors were used in this study. Donors were 47% female and had a mean age of 67.2 ± 9.4 years.

2.3.1.2. Stratum corneum. Stratum corneum samples were prepared by floating heat-separated epidermal membranes on an aqueous solution of 0.0001% w/v trypsin and 0.5% w/v sodium hydrogen carbonate at 37°C for 12 h. The stratum corneum was separated from digested epidermal tissue by swabbing and was rinsed with water several times. The washed sheets were dried on a PTFE-coated wire mesh at room temperature and then washed with ice-cold acetone for 10 s and stored desiccated until required.

2.3.1.3. Silastic membranes. Silastic membranes were washed in water and boiled for 10 min in distilled water. They were then rinsed once more and left to soak overnight in distilled water (Bond, 1986).

2.3.1.4. Skin-silastic membrane sandwiches. The occlusion simulation technique (Tiemessen et al., 1989a,b) was used. Small squares of epidermal membranes or stratum corneum were placed on top of silastic membranes covered with a thin layer of silicone adhesive (to ensure good contact of the skin with the silastic membrane). The skin was then covered with another piece of sticky silastic membrane. Double silastic membranes (with no skin enclosed) were prepared to act as controls.

2.3.1.5. Snake skin

The epidermis of *Elaphe obsoleta* obtained at shedding was sealed in a polythene bag at room temperature. Before diffusion studies samples of snake skin were floated on 0.002% w/v aqueous sodium azide solution to hydrate for 3 days.

2.3.2. Permeation experiments

Permeation experiments were performed at 32 ± 1°C using an automated diffusion apparatus with 24 stainless-steel cells (Akhter et al., 1984). Human epidermal membranes, model non-porous membranes (silastic sheeting), skin-silastic sandwiches and snake skin membranes were mounted in the diffusion cells having a cross-sectional area of 0.126 cm^2. The upper surfaces of the membranes were exposed to unstirred saturated solutions of OE in different ethanol/water co-solvent systems and the receptor fluid was the ethanol/water mixture corresponding to that in the donor compartment. Receptor solution was pumped continuously through the receptor compartment at a rate of 2 ml h^{-1}. Receptor solution leaving the cells was collected in scintillation vials containing 5 ml scintillant fluid, OptiPhase, HiSafe 3 (LKB Scintillation Products, UK), and held on a carousel which automatically changed vials at pre-set time intervals.

The concentration of radiolabelled OE in the samples was analysed by liquid scintillation counting using a Packard, Tri-Carb-1600 TR scintillation counter.

2.4. Partition and uptake studies

Acetone rinsed dry stratum corneum and silastic membrane discs were weighed and hydrated in 0.002% w/v aqueous sodium azide solution. After 72 h the samples were floated flat onto tissue paper, blotted dry, reweighed and equilibrated with near saturated solutions of radiolabelled OE in different ethanol/water co-solvent systems for 48 h. The stratum corneum discs were blotted dry to remove excess drug solutions, weighed wet and dissolved in tissue solubiliser (Soluene-350 Packard, Meriden, USA) then stored overnight to allow chemiluminescence to subside. The drug in silastic membrane samples was extracted with acetone. The concentration of drug in the membrane and in the donor solution after partitioning was determined by liquid scintillation counting after addition of 5 ml scintillant fluid. The parti-

tion coefficients were calculated by dividing the concentration of drug in the membrane by that in the donor solution.

2.5. Membrane uptake of water and ethanol

Previously weighed hydrated stratum corneum samples and silastic membranes were equilibrated with aqueous ethanol solutions with various weight fractions of [^{14}C]ethanol, and a second set was equilibrated with various weight fractions of ethanol in tritiated water. After 72 h the samples were blotted dry and the concentration of water and ethanol in the membranes and donor solutions determined by liquid scintillation counting.

2.6. Data analysis

The permeability coefficients of OE in the sandwich membrane, epidermal membrane and stratum corneum were calculated using Eq. 1 and 2 (Flynn et al., 1974). The permeability coefficients in the silastic double-membrane were determined for each donor/receptor fluid composition, and the contribution of the silastic double-membrane to the total resistance of the sandwich was separated from the contribution due to the skin using Eq. 2:

$$J = K_p \times C \quad (1)$$

$$\frac{1}{K_{p_s}} = \frac{1}{K_{p_{sand}}} - \frac{1}{K_{p_m}} \quad (2)$$

where J is the pseudo steady state flux of the drug through the sandwich, K_p the permeability coefficient, C the drug concentration in the donor solution, and s, sand, and m relate to skin, sandwich and silastic double membrane, respectively.

3. Results and discussion

In this investigation all the permeation studies were conducted using saturated drug solutions, i.e., with the permeant at its maximum chemical potential. In the absence of any specific solvent/membrane interaction, the flux of OE from each solvent system ideally should be constant (Higuchi, 1960).

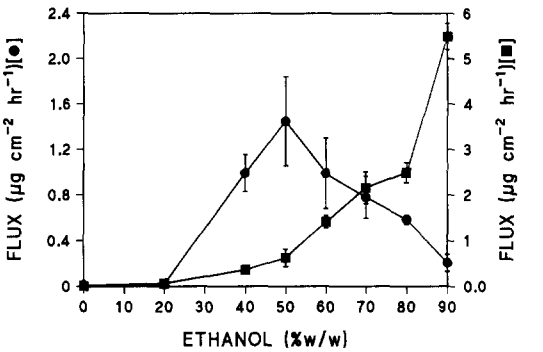

Fig. 1. Fluxes of oestradiol from saturated ethanol/water co-solvent systems (0–90% w/w) through human epidermal membrane (●) and silastic membrane (■). Each point represents the mean of three experiments ± S.E. (bar lines within the size of symbol not shown).

However, the flux of OE across human epidermal membranes from saturated ethanol/water co-solvent systems increased substantially with increasing ethanol concentration in the donor solution from 0.015 ± 0.004 μg cm^{-2} h^{-1} when delivered from pure water up to a maximum flux of 1.45 ± 0.390 μg cm^{-2} h^{-1}, an approx. 95-fold increase in flux. An optimal concentration range of aqueous ethanol producing a maximum in flux was observed between 40 and 60% w/w. At higher ethanol concentrations the OE flux decreased (Fig. 1).

Several studies have reported the effect of ethanol on human or animal skin where a solvent gradient existed across the membranes, i.e., ethanol was present only in the donor solution and the receptor fluid was either water or saline. Optimum enhancement of salicylate ion permeation through human skin has been observed with ethanol volume fractions near 0.63 using saline as receptor fluid (Kurihara-Bergstrom et al., 1990). Hatanaka et al. (1993) reported a marked enhancement of the permeation rate of seven lipophilic drugs including oestradiol, through hairless mouse skin from ethanol concentrations over the range 0–60% v/v, although no such effect was found for hydrophilic drugs (e.g., 5-fluorouracil). Similar to our results, Berner et al. (1989a) reported that an optimal concentration

range of aqueous ethanol (50–70% v/v) in the donor solutions produced a 5–10-fold increase in nitroglycerin flux across human skin and suggested that the enhanced nitroglycerin flux from ethanol concentrations less than 70% was related to the flux of ethanol itself. However, our experimental design of using the same solvent composition in the donor and receptor compartments dictates that no net flux of ethanol exists across the membranes. Consequently, in our studies the drug flux across the membrane is not related to co-solvent component flux.

In contrast to these results, ethanol has been reported to enhance continuously the permeation of oestradiol and other lipophilic permeants through hairless mouse skin over the ethanol/saline fraction range 0.0–1.0 in both the donor and receptor chamber (Ghanem et al., 1987). The same design of the same solvent component in the donor and receptor compartments has been used by Liu et al. (1991) who reported a continuous flux enhancement of saturated oestradiol across human epidermal membrane as a function of ethanol concentration but only for the range 0–75% v/v on both sides of the membrane. These results suggest that ethanol enhances the stratum corneum transport and its own permeation by increasing the respective diffusion coefficients at ethanol concentrations < 50% and by both increasing diffusion coefficient and decreasing membrane activity coefficient at moderate concentrations (50–75% v/v). Additionally, they demonstrated that the permeant flux, in general, is not linear with the co-transported enhancer flux, in contrast to the results of Berner et al. (1989a).

Therefore, in order to clarify the effect of ethanol on OE permeation through human skin, the influence of ethanol on the partition coefficient of OE was investigated. Fig. 2 shows that increasing the ethanol concentration in the donor solution leads to a decrease in the stratum corneum:vehicle partition coefficient. This decrease is inversely correlated to the increased OE solubility in the vehicles tested, in agreement with the result obtained by Pershing et al. (1990) and Ghanem et al. (1987). However, the uptake of OE by stratum corneum increases as the concentration of ethanol in the donor solution increases as shown in Fig. 3. This result suggests that the enhanced flux of OE at concentrations less than 60% shown in Fig. 1 may be related to increased drug solubility in the stratum corneum.

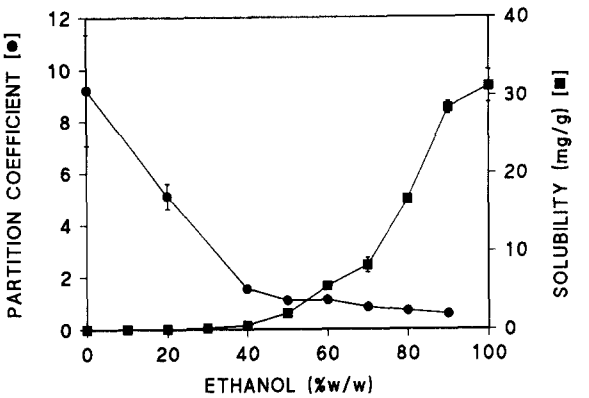

Fig. 2. The effect of different ethanol concentrations on the partition coefficient of oestradiol between the stratum corneum and the ethanol/water co-solvent systems (●). Each point represents the mean of nine experiments ± S.E. Also shown is the saturated solubility of oestradiol in the tested co-solvents (■). Each point is the mean of three determinations ± S.E. (bar lines within the size of symbol not shown).

The effect of ethanol on a heterogeneous membrane such as human skin may be difficult to interpret and to relate mechanistically to skin permeation. One approach for studying the ethanolic effects is to use a homogenous inert

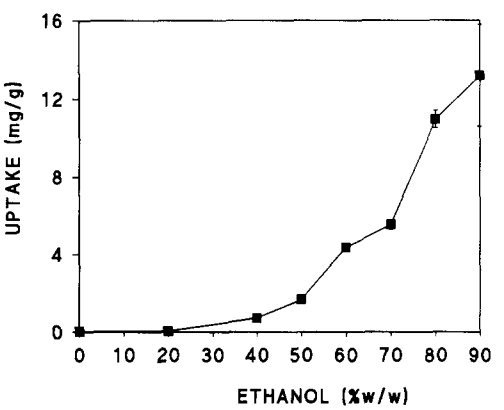

Fig. 3. Uptake of oestradiol from different saturated ethanol/water co-solvent systems by stratum corneum. Each point represents the mean of three experiments ± S.E. (bar lines within the size of symbol not shown).

membrane to check if the ethanol action is particular to human skin. Silastic (polydimethylsiloxane) has been suggested as a model membrane for human percutaneous absorption studies (Flynn and Smith, 1972), and its permeability to a drug such as salicylic acid has been shown to be similar to that of human skin (Cooper, 1984). Nakano and Patel (1970) studied the release of salicylic acid from several ointment bases using silicon rubber membrane as a model for skin. Their in vitro release results exhibited a perfect rank order correlation to published in vivo data for the same system.

The silastic membrane was therefore chosen to study the effects of ethanol on OE flux. As this study is comparative, and the resistance due to the stagnant solvent layer adjacent to the membrane was insignificant in human skin experiments, it was also neglected for the silastic membrane study and the membrane resistance was assumed to be rate-limiting in all experiments.

Unlike human skin, permeation data across the model lipophilic silastic membrane showed a continual increase in OE flux up to 5.48 ± 0.29 $\mu g\ cm^{-2}\ h^{-1}$ at an ethanol concentration of 90% w/w, with no evidence of maximum flux (Fig. 1). Enhanced flux of methylparaben through polydimethylsiloxane membrane was observed from saturated solutions in various ethanol/water concentrations (Twist and Zatz, 1986). At low ethanol concentration, the flux of methylparaben rose linearly with increasing ethanol concentration. At very high ethanol concentrations (mole fraction of 0.8 and above) there was a much greater increase in flux. Ethanol uptake measurements using ethanol/water mixtures revealed that the membrane imbibed the ethanol and the effect became more pronounced as the ethanol activity within the solvent system was increased. The flux increase correlated with the membrane solubility of parabens for these systems; the diffusion coefficient also increased to a small extent relative to a noninteractive solvent.

Ethylene vinyl acetate, another model membrane, gave a similar trend with nitroglycerin in different ethanol/water co-solvent systems and it was concluded that the nitroglycerin permeability increase arose from ethanol interaction with the membrane, i.e., that ethanol must be plasticising this membrane (Berner et al., 1989b).

The partition coefficient and the uptake of OE by silastic membranes (Fig. 4) show similar trends to those obtained with human stratum corneum (Fig. 2 and 3). Thus, from the uptake result, the increase in OE flux through human skin at ethanol concentrations lower than 60% w/w and through silastic membrane at ethanol concentrations between 0 and 90% w/w can be attributed at least in part to the increased solubility of OE in the membranes.

If the increased OE solubility in the stratum corneum is the only factor in increasing the OE

Table 1
Oestradiol flux, uptake and ratios relative to water from different ethanol/water co-solvent systems for human skin and silastic membranes

Human skin					Silastic membrane			
Ethanol (% w/w)	Flux ($\mu g\ cm^{-2}\ h^{-1}$)	Flux ratios	Uptake (mg/g)	Uptake ratios	Flux ($\mu g\ cm^{-2}\ h^{-1}$)	Flux ratios	Uptake (mg/g) ($\times 10^{-3}$)	Uptake ratios
0	0.015 ± 0.005	1.00	0.032 ± 0.001	1.00	0.004 ± 0.001	1.00	0.851 ± 0.161	1.00
20	0.019 ± 0.002	1.29 ± 0.05	0.045 ± 0.006	1.38 ± 0.19	0.067 ± 0.012	16.4 ± 3.89	2.894 ± 0.213	3.40 ± 0.14
40	0.993 ± 0.163	45.4 ± 6.90	0.717 ± 0.150	22.2 ± 4.64	0.362 ± 0.054	89.1 ± 11.3	15.80 ± 1.631	18.6 ± 1.11
50	1.450 ± 0.393	95.5 ± 25.6	1.670 ± 0.078	29.9 ± 2.25	0.627 ± 0.191	163 ± 34.1	33.80 ± 2.050	39.7 ± 13.9
60	0.990 ± 0.310	79.1 ± 22.1	4.350 ± 0.127	135 ± 3.93	1.415 ± 0.140	329 ± 38.3	56.3 ± 16.00	68.4 ± 3.50
70	0.780 ± 0.195	50.5 ± 12.0	5.540 ± 0.237	172 ± 7.34	2.160 ± 0.350	399 ± 5.52	89.80 ± 7.00	82.0 ± 5.8
80	0.596 ± 0.040	38.5 ± 3.52	10.96 ± 0.445	339 ± 13.8	2.495 ± 0.220	580 ± 50.2	104.1 ± 40.00	125 ± 19.9
90	0.206 ± 0.075	13.6 ± 4.96	13.20 ± 2.598	409 ± 80.2	5.489 ± 0.290	1275 ± 66.9	255.0 ± 68.00	299 ± 30.7

Results are presented as mean ± standard error of mean ($n = 3$–5).

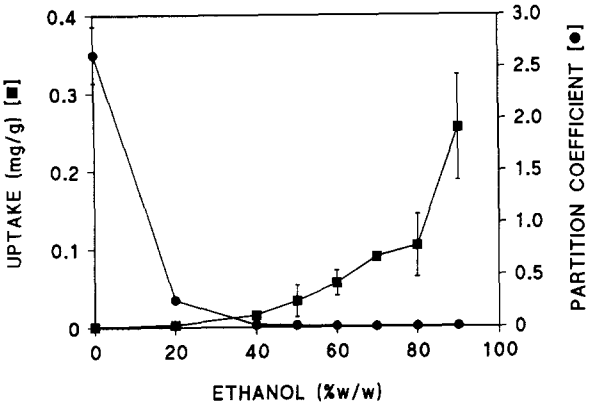

Fig. 4. The effect of different ethanol concentrations on the partition coefficient (●) and uptake (■) of oestradiol by the silastic membrane. Each point represents the mean of three experiments ± S.E. (bar lines within the size of symbol not shown).

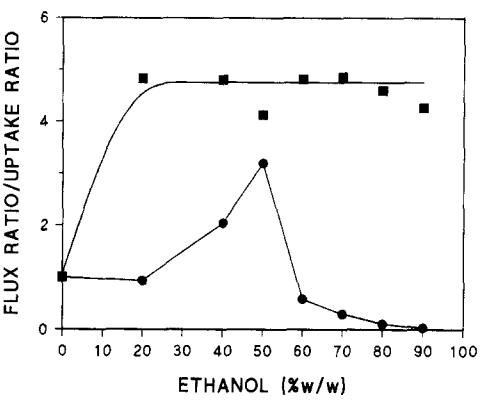

Fig. 5. Flux ratio/uptake ratio of oestradiol from different saturated ethanol/water co-solvent systems; (●) human skin, (■) silastic membrane.

permeation, the enhanced flux of OE, i.e., flux ratio (Fr) and the enhanced uptake, i.e., uptake ratio (Ur) from different ethanol concentrations relative to that from water should be linearly related, and a plot of Fr/Ur of OE against ethanol concentrations should be a horizontal line at unity. Therefore, to examine this phenomenon the enhancement of OE flux (Fr) and uptake (Ur) from different ethanol concentrations relative to the flux and uptake from water was calculated (Table 1) and Fr/Ur values for both human skin and silastic membrane data were plotted against ethanol concentrations (Fig. 5). The human skin results showed that, up to ethanol concentrations of 20% w/w, the Fr/Ur was essentially constant and equal to unity, indicating that the principal mechanism of enhanced flux is increased drug solubility in the stratum corneum. Further increase in ethanol from 20 to 50% w/w led to an upward deviation from unity, indicating that ethanol affected the physicochemical properties of the stratum corneum matrix so as to reduce its barrier function besides increasing drug uptake. At higher ethanol concentrations the Fr/Ur ratio decreased because of the dehydration effect. Ethanol is a solvent known to modify skin barrier properties. A variety of mechanisms has been proposed to explain penetration enhancement by ethanol via non-partitioning effects, including lipid fluidisation and pore formation in stratum corneum (Ghanem et al., 1987; Higuchi et al., 1987), lipid disordering (Rowe, 1985), lipid extraction (Bommannan et al., 1991), and perturbation of the lipoidal barrier at low ethanol concentration including conformational changes of keratinised protein and partial lipid extraction (Kurihara-Bergstrom et al., 1990). In our laboratory, fluidisation of the lipids has been observed after treatment of stratum corneum with different concentrations of ethanol, as assessed by differential scanning calorimetery (unpublished data).

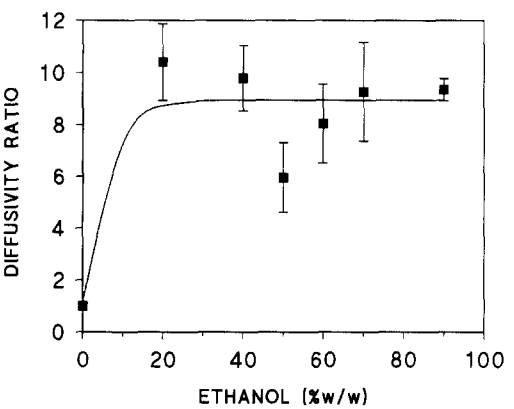

Fig. 6. Diffusivity ratio of oestradiol from different saturated ethanol/water co-solvent systems through silastic membrane relative to diffusivity from water. Each point represents the mean of three or more experiments ± S.E.

Silastic membrane (Fig. 5) showed an increase in Fr/Ur of about 4-fold in the presence of ethanol concentrations of 20–90% w/w, this feature suggesting changes in physicochemical properties of the membrane and thus enhanced diffusivity of oestradiol. An approximate assessment of the apparent diffusion coefficients (D) of oestradiol through silastic membrane from water and ethanol/water mixtures was calculated using measured permeability coefficients (K_p), partition coefficients (P_c) and thickness of the membrane (h) applying the following equation:

$$D = \frac{K_p \times h}{P_c} \quad (3)$$

Fig. 6 shows that diffusion coefficient ratios of OE through silastic from ethanol concentration 20–90% w/w relative to that from water were about 8; the 2-fold difference from the value of Fr/Ur is acceptable in the light of the approximations made in these types of experiments. Although the diffusivity data are scattered, an analysis of variance shows no significant differences between the means of the diffusion coefficients derived from ethanol concentration 20–90% w/w ($p \leq 0.05$). This constant diffusivity at different ethanol concentrations may be explained by the results of ethanol and water uptake experiments on silastic membranes. Fig. 7 shows that ethanol uptake by silastic membrane was only slightly changed (8–12 mg/g) from ethanol concentration 20–90% w/w with a sudden increase from pure ethanol. Water uptake was only 5 mg/g from pure water and decreased to 2 mg/g in the presence of ethanol.

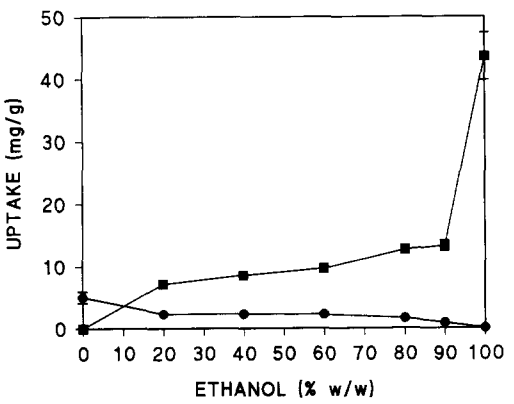

Fig. 7. Uptake of water (●) and ethanol (■) from different ethanol/water systems by silastic membranes. Each point represents the mean of three experiments ± S.E. (bar lines within the size of symbol not shown).

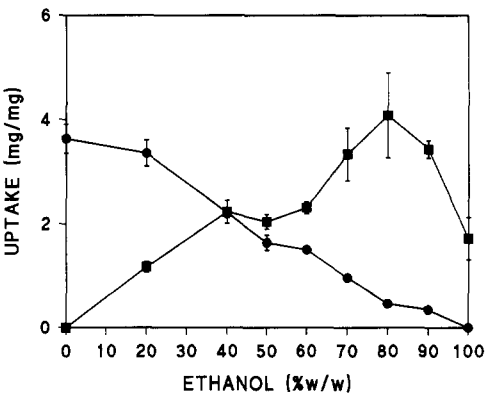

Fig. 8. Uptake of water (●) and ethanol (■) from different ethanol/water systems by stratum corneum. Each point represents the mean of three experiments ± S.E. (bar lines within the size of symbol not shown).

Fig. 1 shows a decrease of OE enhanced flux through human skin at ethanol concentrations higher than 60% w/w and it is necessary to explain this effect. Ethanol may remove water from stratum corneum and thus decrease its hydration level. It is well known that the percutaneous absorption of most substances is increased by raising the water content of stratum corneum and its dehydration should therefore decrease the permeation of such substances. Blank et al. (1984) estimated that the diffusivity of water within stratum corneum halved as relative humidity dropped from 93 to 46%. The hydration of human epidermis can induce manifold increases in its permeability and it has been shown that permeability to water can be increased 8-fold through hydration (Scheuplein and Ross, 1973), and 20-fold enhancement was seen with cortisone when dry stratum corneum was exposed to water vapour (Scheuplein et al., 1969). In vivo the use of occlusive dressings reportedly increased the absorption of steroids as much as 100-fold (McKenzie and Stoughton, 1966) although this was probably an overestimate. Therefore, although OE solubility in the skin was increased further with increasing

ethanol concentrations above 60% w/w it is possible that the dehydration effects of ethanol on the stratum corneum may limit and even decrease the OE flux as predicted only from stratum corneum partitioning data. This dehydration effect may also overshadow the suggested effect of lipid extraction by ethanol at higher concentration (Kurihara-Bergstrom et al., 1990; Bommannan et al., 1991).

Therefore, to investigate the decrease of OE flux through human skin at ethanol concentrations higher than 60% w/w, the water and ethanol uptake by stratum corneum from different ethanol/water systems was determined. Fig. 8 shows that up to nearly 4-times its dry weight in water was taken up by stratum corneum. This large water uptake was nearly constant up to 20% w/w ethanol, and then decreased approximately linearly with increased ethanol concentration. At a concentration of ethanol between 50 and 60% w/w where the maximum OE flux was observed, the water uptake was between 150 and 160% w/w, i.e., it was decreased by 45% compared with that from the pure water. At 90% w/w ethanol, the water content of stratum corneum was only 35% w/w per dry stratum corneum weight. On the other hand, the stratum corneum ethanol uptake increased with increasing ethanol concentration in the bathing solutions to a maximum at 80% w/w ethanol. With neat ethanol, uptake of ethanol by stratum corneum decreased in spite of the increase in ethanol driving force. This may be a consequence of dehydration of the stratum corneum. From these results we suggest that the decrease in the OE flux at higher ethanol concentration is due to a dehydration effect of ethanol on the stratum corneum, thereby increasing the barrier properties of the skin, and that this effect overcomes the further increase in OE delivered to the skin.

To provide further evidence for this hypothesis another experimental design was developed where the water content of skin could be controlled even though the ethanol/water ratio varied in the bathing solution. Recently a new in vivo model (in vivo occlusion simulation technique) has been developed to study passive diffusion through human tissue, in particular stratum corneum. The

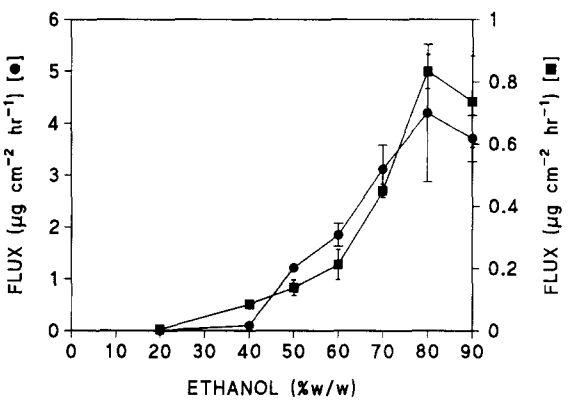

Fig. 9. Oestradiol flux through hydrated epidermal membrane (●) and dry stratum corneum (■) in the sandwich model from different saturated ethanol/water co-solvent systems. Each point represents the mean of three experiments ± S.E. (bar lines within the size of symbol not shown).

hydration level of the human stratum corneum membrane during a permeation experiment is controlled by sandwiching skin between two silastic membranes. Under these conditions only a slow and steady increase in stratum corneum water content from 45 to 54% w/w during a 16 h experiment was observed (Tiemessen et al., 1989b). This slight change would be acceptable for our purpose. The diffusional resistance of silastic membrane is relatively small compared with that of human skin. It can be determined by a separate experiment and the value subtracted from that for the composite membrane to yield the resistance for skin (Eq. 2).

Fig. 9 shows that the flux through fully hydrated epidermal membrane inside the sandwich model increased continuously with increasing ethanol concentrations in the donor solutions. No significant decrease in the flux was observed at higher ethanol concentrations as was previously seen with the uncontrolled skin (Fig. 1). The apparent slight fall in flux from 80 to 90% w/w ethanol was within the error bars.

Work in our laboratory has shown that there is no significant difference in OE permeation through the stratum corneum, epidermal and full thickness skin and we concluded that the intact stratum corneum is the main barrier to the OE permeation (Williams and Barry, 1991). There-

fore, the permeation of OE through dry stratum corneum in the sandwich model was also studied, and the results showed a similar trend to that obtained with the hydrated epidermal membranes (Fig. 9). Thus, when the hydration level in the stratum corneum was not allowed to drop, then the flux also did not fall. This procedure thus provided further evidence of the importance of skin dehydration in decreasing OE flux in unprotected stratum corneum.

The sandwich technique has been used to quantify the influence of water content of stratum corneum on its permeability to nitroglycerin. A 6-fold increase in stratum corneum permeability was observed as its water content changed from 17.5 to 45% w/w (Tiemessen et al., 1989a). Thus, it is interesting to note that in our study the penetration rate of OE through the fully hydrated epidermal membrane was, as expected, much greater than that for the dry stratum corneum when both are present in the sandwich model. For example there was about a 9-fold increase in OE flux from the 50% w/w ethanol through the fully hydrated epidermal membrane when compared with dry stratum corneum.

So far, the experimental approach has been to use primarily human skin and to probe the mechanisms of the ethanol effect by also investigating fluxes and uptakes in silastic membranes and composite membranes. It would be useful to include another membrane for comparison, but one of biological origin. Such a membrane of current interest is shed snake skin. The American black rat snake, *E. obsoleta*, has been used previously as a model membrane for human skin in vitro permeation studies and was therefore selected (Roberts, 1986; Higuchi and Konishi, 1987; Millard and Barry, 1989; Itoh et al., 1990; Rigg and Barry, 1990).

Snake skin structure is similar to that of human stratum corneum in that it contains cornified tissue with phospholipids instead of ceramides. However, snake skin has a low ability to take up water compared with human stratum corneum. It was therefore of interest to see if this membrane would behave similarly to human skin with regard to the ethanol effect on the OE flux. The influence of ethanol on the flux of oestradiol across

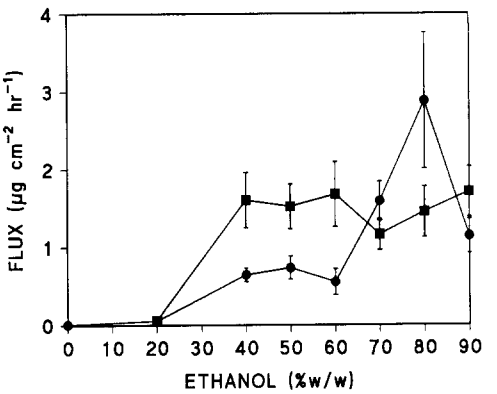

Fig. 10. Oestradiol flux through dorsal (●) and ventral (■) snake skin from different saturated ethanol/water co-solvent systems. Each point represents the mean of three or more experiments ± S.E. (bar lines within the size of symbol not shown).

the scales of both dorsal and ventral shed snake skin of *E. obsoleta* was therefore examined. Fig. 10 shows that the flux of oestradiol through dorsal snake skin increased with increasing ethanol concentrations in the donor solution with a maximum flux at 80% w/w ethanol. Compared with human skin the maximum flux of OE thus appeared at a higher ethanol concentration. This difference from human skin may be due to the lower water content of dorsal snake skin which was only 48% w/w. This value was obtained after 3 days soaking in water while for human skin soaked for the same period the water uptake was about 400%. Thus, for dorsal snake skin the dehydration effect of ethanol only showed up at ethanol concentrations in excess of 80% w/w. On the other hand, ventral snake skin, which had even less ability for water uptake (35% w/w) and thus subsequent dehydration by ethanol, showed an increased flux of OE with increasing ethanol concentration up to 40% w/w. At higher ethanol concentrations, the permeation of oestradiol through ventral snake skin was essentially independent of ethanol content in the bathing solutions.

Although human skin is the most relevant to clinical considerations, some investigators have used animal skin as a model for human in vitro penetration studies because of the limited avail-

ability of human skin. Snake skin is one such model membrane for which there are various reports in the literature in terms of its similarity and dissimilarity to the permeability of human skin. In the study of Itoh et al. (1990), the permeation of various compounds through snake skin was determined and compared with the reported values for human skin. The results showed that permeation of phenol, *m*-cresol, methylparaben and 11α-hydroxyprogesterone was 1.5–2-fold lower than that for human skin, and the permeation of corticosterone was approximately twice that for human skin. Rigg and Barry (1990) reported similar permeabilities of dorsal snake skin and human skin for water, but approximately one order of magnitude higher for the permeation of 5-fluorouracil through snake skin compared with that for human skin. Similarity of salicylic acid permeation through snake skin and human breast, thigh, back and leg lower skin has been reported (Harada et al., 1993).

In the present study the flux values of OE through dorsal and ventral snake skin from different ethanol/water mixtures were compared with those obtained from human skin. As detailed above, the flux of OE from different ethanol/water solutions through dorsal and ventral snake skin differ. Data from dorsal snake skin up to 60% w/w ethanol tended to show flux values 2-fold lower than that from human skin. On the other hand the flux through ventral snake skin over the ethanol range 20–80% w/w was 1.5–3-fold higher than for human skin and up to 8-fold higher at 90% w/w ethanol. These results thus show some similarity in the permeability of snake skin and human skin especially when there is no specific solvent effect on the membranes. Data from snake membranes tended to underestimate the effect of enhancers on human skin (Rigg and Barry, 1990).

From all the results of this study considered together we can reach a general conclusion. Enhanced permeation of oestradiol across human skin in vitro in the presence of ethanol concentrations lower than 60% w/w can be attributed at least in part to an increased solubility of drug in the stratum corneum. The remaining enhancement for this concentration range arises from a direct action of the ethanol on the stratum corneum whereby its diffusional resistance is diminished. The decrease in oestradiol flux at ethanol concentrations above 60% can be explained by a dehydration effect of ethanol on the stratum corneum. The lipophilic silastic membrane and snake skin employed as models for human skin are not reliable for assessing the effects of ethanol on human percutaneous absorption, but their use in valuable in elucidating the mechanism of action of ethanol as an enhancer.

References

Akhter, S.A., Bennett, S.L., Waller, I.L. and Barry, B.W., An automated diffusion apparatus for studying skin penetration. *Int. J. Pharm.*, 21 (1984) 17–26.

Barry, B.W., *Dermatological Formulations: Percutaneous Absorption*, Dekker, 1983.

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

Berner, B., Otte, J.H., Mazzenga, G.C., Steffens, R.J., Ethanol:water mutually enhanced transdermal therapeutic system: I. Nitroglycerin solution properties and membrane transport. *J. Pharm. Sci.*, 78 (1989b) 314–318.

Blank, I.H., Cutaneous barrier. *J. Invest. Dermatol.*, 45 (1965) 249–256.

Blank, I.H., Moloney, J., III, Emsilie, A.E., Simon, I. and Apt, C., The diffusion of water across the stratum corneum as a function of its water content. *J. Invest. Dermatol.*, 82 (1984) 188–194.

Bommannan, D., Potts, R.O and Guy, R.H., Examination of the effect of ethanol on human statum corneum in vivo using infrared spectroscopy. *J. Controlled Release*, 16 (1991) 299–304.

Bond, J.R., Human skin and model membranes for permeation studies. Ph.D.Thesis, University of Bradford (1986).

Campbell, P.S. and Chandrasekaran, S.K., *US Patent 4,379,454*, 1983.

Cooper, E.R., Increased skin permeability for lipophilic molecules. *J. Pharm. Sci.*, 73 (1984) 1153–1156.

Flynn, G.L. and Smith, R.W., Membrane diffusion: II. Influence of physical adsorption on molecular flux through heterogeneous dimethylpolysiloxane barriers. *J. Pharm. Sci.*, 60 (1972) 1788–1796.

Flynn, G.L., Yalkowsky, S.H and Roseman, T.J., Mass transport phenomena and models: theoretical concepts. *J. Pharm. Sci.*, 63 (1974) 479–510

Gale, R.M. and Berggen, R.G., *US Patent 4615699*, 1986.

Gale, R.M., Lee, E.S., Taskovich L.T. and Yum, S.I., *US Patent 4588580*, 1986.

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

Harada, K., Murakami, T., Kawasaki, E., Higashi, Y., Yamamoto, S. and Yata, N., In vitro permeability to salicylic acid of human, rodent and snake skin. *J. Pharm. Pharmacol.*, 45 (1993) 414–418

Harrison, S.M., Barry, B.W. and Dugard, P.H., Effect of freezing on human skin permeability. *Int. J. Pharm.*, 36 (1984) 261–262.

Hatanaka, T., Shimyama, M., Sugibayashi, K. and Morimoto, Y., Effect of vehicle on the skin permeability of drugs: polyethylene glycol 400-water and ethanol- water binary solvents. *J. Controlled Release*, 23 (1993) 247–260.

Higuchi, T., Physical chemical analysis of percutaneous absorption process from creams and ointments. *J. Soc. Cosmet. Chem.*, 11 (1960) 85–97.

Higuchi, T. and Konishi, R., In vitro testing and transdermal delivery, Proceeding of the 2nd Transdermal Therapeutic Symposium. *Ther. Res.*, 6 (1987) 280–288.

Higuchi, W.I., Rohr, U.D., Burton, S.A., Liu, P., Fox, J.L., Ghanem, A.H., Mahmoud. H., Borsadia, S. and Good, W.R., Effect of ethanol on the transport of β-estradiol in hairless mouse skin. Comparison of experimental data with a new theoretical model. In *Controlled Release Technology, Pharmaceutical Applications*, ACS Symp. Ser. 348, American Chemical Society, Washington, 1987, pp. 232–240

Itoh, T., Xia, J., Magavi, R., Nishihata, T., Rytting, J.H., Use of shed snake skin as model membrane for in-vivo percutaneous penetration studies: Comparison with human skin. *Pharm. Res.*, 7 (1990) 1042–1047.

Kligman, A.M. and Christophers, E., Preparation of isolated sheet of human stratum corneum. *Arch. Dermatol.*, 88 (1963) 70–73.

Knutson, K., Krill, S.L. and Zhang, J., (1990) Solvent mediated alterations of the stratum corneum. *J. Controlled Release*, 11, 93–103.

Kurihara-Bergstrom, T., Knutson, K., DeNoble, J. and Goates C.Y., Percutaneous absorption enhancement of ionic molecules 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.

McKenzie, A. and Stoughton, R., Methods for comparing percutaneous absorption of steroids. *Arch. Dermatol.*, 86 (1966) 608–610.

Millard, J.L. and Barry, B.W., Iontrophoresis – a comparative study of human skin and snake skin. *J. Pharm. Pharmacol.*, 41 (1989) 136P.

Mollgaard, B. and Hoelgaard, A., Permeation of estradiol through the skin – Effect of vehicle. *Int. J. Pharm.*, 15 (1983) 185–197.

Nakano, M. and Patel, N.K., Release, uptake and permeation behavior of salicylic acid ointment base. *J. Pharm. Sci.*, 59 (1970) 985–988.

Obata, Y., Takayama, K., Maitani, Y., Machida, Y. and Nagai, T., Effect of ethanol on skin permeation of nonionized and ionized diclofenac. *Int. J. Pharm.*, 89 (1993) 191–198.

Pershing, L.K, Lambert, L.D. and Knutson, K., Mechanism of ethanol enhanced oestradiol permeation across human skin in-vivo. *Pharm. Res.*, 7 (1990) 170–175.

Rigg, P.C. and Barry, B.W., Shed snake skin and hairless mouse skin as model membranes for human skin during permeation studies. *J. Invest. Dermatol.*, 94 (1990) 235–240.

Roberts, J.B., Use of squamate epidermis in percutaneous absorption studies: Review. *J. Toxicol. Cut. Ocul. Toxicol.*, 5 (1986) 319–332.

Rowe, E.S., Thermodynamic reversibility of phase transitions. Specific effect of alcohols on phosphatidylcholines. *Biochim. Biophys. Acta*, 813 (1985) 321–330.

Scheuplein, R.J. and Ross, L., Effect of surfactant and solvent on the permeability of epidermis. *J. Soc. Cosmet. Chem.*, 21 (1973) 853–873.

Scheuplein, R.J., Blank, I.H., Brauner, G.J. and Macfarlane, D.J., Percutaneous absorption of steroids. *J. Invest. Dermatol.*, 52 (1969) 63–70.

Tiemessen, H.L.G.M., Boddé, H.E. and Junginger, H.E., A silicone membrane sandwich method to measure drug transport through isolated human stratum corneum having fixed water content. *Int. J. Pharm.*, 56 (1989a) 87–94.

Tiemessen, H.L.G.M., Boddé, H.E., Mollee, H. and Junginger, H.E., A human stratum corneum-silicone membrane sandwich to simulate drug transport under occlusion. *Int. J. Pharm.*, 53 (1989b) 119–127.

Twist, J.N. and Zatz, J.L., Influence of solvents on paraben permeation through idealized skin model membranes. *J. Soc. Cosmet. Chem.*, 37 (1986) 429–444.

Williams, A.C. and Barry, B.W., The enhancement index concept applied to terpene penetration enhancers for human skin and model lipophilic (oestradiol) and hydrophilic (5-flurouracil) drugs. *Int. J. Pharm.*, 74 (1991) 157–168.

Yum, S.I., Lee, E.S., Taskovich, L. and Theeuwes, F., Mechanism of ethanol as flux enhancer for drug permeation through human skin. *Proc. Int. Symp. Controlled Release Bioact. Mater.*, 14 (1987) 103–104.