Rate Control in Transdermal β-Estradiol Reservoir Membrane Systems: The Role of Membrane and Adhesive Layer

Ralf Altenburger,[1,3] Uwe D. Rohr,[1,2] and Thomas Kissel[1]

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Purpose. The aim of our study was to clarify the kinetic performance of a membrane controlled reservoir system (MCRS) for β-estradiol (E_2) under *in vitro* conditions by determination of the role of membrane and adhesive layer on E_2 flux control.

Methods. E_2 and ethanol fluxes across EVA membrane or membrane coated with adhesive from saturated solutions in defined ethanol/PBS mixtures were measured in the symmetric and asymmetric configuration. Physicochemical parameters of the EVA membrane were determined.

Results. The E_2 flux across the 9% EVA membrane steadily increased with increasing ethanol concentrations in both configurations, due to enhanced uptake of E_2 by the polymer and increasing membrane diffusivity. Permeation across the EVA membrane coated with an adhesive layer in the symmetric and asymmetric configuration increased up to maximum values of 0.80 ± 0.14 $\mu g \times cm^{-2} \times h^{-1}$ and 0.37 ± 0.02 $\mu g \times cm^{-2} \times h^{-1}$, respectively, at 62.5% (v/v) ethanol. The fluxes then decreased with further increase in the volume fraction of ethanol due to a dramatically reduced permeability of the adhesive layer. For the asymmetric case, a linear dependence of E_2 on ethanol fluxes was observed.

Conclusions. The E_2 flux from MCRS is strictly dependent on reservoir ethanol concentrations, whereas the adhesive layer represents the rate controlling barrier at high ethanol levels (>70% v/v).

KEY WORDS: estradiol; ethanol; transdermal delivery; reservoir system; ethylene vinyl acetate membrane; rate control.

INTRODUCTION

The advantage of transdermal delivery of β-estradiol (E_2) over other application routes, such as the oral delivery for estrogen replacement therapy, has been shown by several investigators (1,2). Transdermal application avoids the first pass effect, thereby reducing the required dose, providing more physiologic E_2 plasma levels, and no inducing side-effects such as an overproduction of unwanted hepatic proteins (3).

Currently, two different types of transdermal patches are used for estrogen replacement therapy. Firstly: Patches based on a matrix technology, where the drug is uniformly dispersed in an adhesive polymeric matrix (4,5,6). Secondly: Membrane controlled reservoir systems (MCRS), where a reservoir contains a solution of E_2 and a non-porous membrane supposedly controlling the input rate of E_2 (3).

Although designed for the same therapeutic concept, both types of transdermal systems (TDS) differed significantly in their pharmacokinetic profile. In several clinical studies, MCRS showed significantly higher fluctuations in E_2 plasma level and a peak at 30 to 40 hours in postmenopausal women compared to matrix TDS, which yielded relative constant infusion-like profiles (6,7).

Therefore, the release kinetic of E_2-TDS needs to be clarified to relate patch design to the pharmacokinetic behavior of these systems. We have recently shown that the transdermal flux out of MCRS across excised human skin increases up to a maximum value at 30 to 40 hours followed by a decrease in flux rate (8). This non-constant input rate of E_2 from the MCRS is possibly related to the patch design.

The commercially available E_2-MCRS consists of a reservoir containing a gelled solution of E_2 in 95% (v/v) ethanol. The rate controlling membrane, composed of ethylene-vinylacetate copolymer (EVA) with a vinyl acetate content of 9%, is attached to the skin by an adhesive layer based on a mixture of polyisobutene and light mineral oil (3,9). Ethylene vinyl acetate copolymer membranes have been used in several MCRS, because it is easy to modify their permeability by adjusting the vinyl acetate content (10). The changes in permeability are related to changes in the glass transition temperature and crystallinity of EVA. The membrane used here showed a cristallinity of ca. 50% (10).

Since ethanol is concomitantly released with E_2 from the patch (11), the ethanol concentration in the reservoir steadily decreases leading to non linear release kinetics. *In vitro* experiments revealed that the permeation of E_2 through membrane, adhesive and excised human skin as a function of donor ethanol concentration increased up to a maximum value at ethanol levels of about 50 to 60% (v/v), indicating a strong influence of reservoir ethanol concentrations on the transdermal E_2 input rate (8).

While there are several reports discussing the influence of ethanol on permeation of E_2 (12–15) and other drug substances like Levonorgestrel (16) or salicylate ions (17) across the skin, there is only scant information available on the influence of ethanol on membranes and adhesives used in MCRS.

Systematic investigations on the effect of ethanol reservoir contents on transdermal drug flux out of MCRS are important, because ethanol is the most commonly used co-solvent in these TDS. It has a relatively good solubilization capacity and flux enhancement activity for a wide range of compounds (18) and is currently used in at least three commercially available transdermal drug delivery systems: estradiol (3), nitroglycerin (19) and fentanyl (20). The aim of this study was to elucidate the role of the involved barriers, namely EVA membrane, adhesive layer and skin in controlling the E_2 flux from a MCRS into the body, to correlate design parameters to the kinetic performance of E_2-MCRS.

MATERIALS AND METHODS

Materials

Estradiol (Sigma Chemical Co., St. Louis, USA) and ethanol 96% (v/v) (Lenz Chemie, Westerburg, Germany) were used

[1] Department of Pharmaceutics and Biopharmacy, Philipps-University, Marburg, Germany.

[2] Zentrum der Inneren Medizin, Klinikum der Johann Wolfgang Goethe Universität, Frankfurt am Main, Germany.

[3] To whom correspondence should be addressed. (e-mail: altenbur@mailer.uni-marburg.de)

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as received. The Estraderm®TTS transdermal delivery system (Batches: 312800 and 313100) contained 2.0 mg of E_2 and was commercially obtained. Ethylene vinyl acetate membrane 9% (w/w) vinyl acetate (No. 9702 Cotrans®) with a thickness of 50 μm was a gift from 3 M Medica, Borken, Germany.

Solubility of E_2 in Ethanol/PBS Mixtures

Excess amounts of E_2 were added to 10 ml of defined ethanol/PBS co-solvent systems from 0 to 96% (v/v) in glass vials. The suspensions were stirred using magnetic bar stirrers for 72 hours in a water bath at 37°C. The saturated solutions were then filtered through Polycarbonate membrane filters (pore size: 0.2 μm, Schleicher & Schuell, Dassel, Germany). The concentration in the filtrate was determined by HPLC after appropriate dilution with ethanol.

E_2 Partition Coefficient into Membranes

Weighed EVA membrane discs with a surface area of 3.14 cm^2 and a thickness of 50 μm were placed in saturated solutions of E_2 in defined ethanol/PBS mixtures from 0 to 96% (v/v). After 72 hours of incubation at 37°C the discs were carefully rinsed with ethanol to remove the excess of drug adsorbed to the surface and blotted dry. The membranes were then quantitatively extracted for 72 hours in ethanol of 96% (v/v) at 37°C. Extracts and donor solutions were analyzed for E_2 by HPLC after appropriate dilution with ethanol. Partition coefficients were calculated from the E_2 concentration in the membrane and the donor solution by the following equation: K = [membrane]/[solution].

Ethanol Uptake into Membranes

Exactly weighed discs of EVA membrane were equilibrated with 2 ml of defined ethanol/PBS mixtures from 25 to 96% (v/v) at 37°C. After 72 h, the discs were removed from the solutions and blotted dry. The membranes were then quantitatively extracted in distilled water for 72 h at 37°C. The amount of ethanol desorbed from the membrane was determined using a HPLC method described below.

EVA Membrane Permeation of E_2 and Ethanol

The E_2 flux through the EVA membrane in dependence on donor ethanol concentrations was studied in a two-chamber diffusion cell set up at 37°C. The membrane was mounted between the two well stirred diffusion cell halves, each having a volume of 4 ml and 0.77 cm^2 in effective diffusion area. Two different configurations were used: For both configurations the donor compartment consisted of a saturated solution of E_2 in defined ethanol/PBS co-solvent systems from 0 to 96% (v/v).

For the symmetric case, the receptor contained a mixture of ethanol and PBS of the same composition as used in the donor. Here only the permeated amount of E_2 was determined.

For the asymmetric case, the receptor consisted of PBS and permeated amounts of E_2 and ethanol were simultaneously determined. The effect of ethanol diffusion on alcohol concentrations in both compartments can be neglected, because firstly the ethanol/PBS volume fraction in the donor phase can be judged as nearly constant within experimental time in the side-by-side *in vitro* diffusion cell, and secondly the diffused amount of ethanol into the acceptor phase is so low, that pure PBS solution can still be assumed.

The receptor medium was periodically changed to maintain sink conditions. Samples of 1 ml were withdrawn from the receiver compartment and replaced with fresh receptor solution. Samples were assayed for E_2 and ethanol by HPLC. Steady state flux rates were determined based on linear regression analysis of total amount permeated versus time curves.

EVA Membrane and Adhesive Permeation

The experimental conditions used for studying the permeation kinetics of E_2 and ethanol across EVA membrane coated with adhesive were the same as outlined above, except that the membrane with attached adhesive layer was used in place of the EVA membrane. The membrane with adhesive was isolated from the commercially available MCRS with the help of a pair of scissors. In this investigation, the donor half-cell faced the membrane while the receiver faced the adhesive layer.

HPLC Analysis

E_2 was assayed by a reverse phase HPLC method previously described (8). Briefly, a LiChrospher®100 RP 18 (5 μm, 250 × 4.6 mm i.d., Merck KGaA) was used as analytical column maintained at 30°C. The mobile phase consisted of acetonitrile/water (60:40) at a flow rate of 1.0 ml/min. E_2 was detected with a fluorescence spectrophotometer set to an excitation wavelength of 225 nm with no emission filter used. The detection sensitivity was 0.1 μg/ml. Ethanol concentrations were measured using a high performance liquid chromatographic system (Merck-Hitachi L 6200 A, Merck KGaA, Darmstadt, Germany) equipped with an AS 2000 A autosampler. A LiChrospher®100 RP 18 (5 μm, 125 × 4,6 mm i.d., Merck KGaA) was used as analytical column maintained at 30°C. The mobile phase used was distilled water at a flow rate of 1.0 ml/min. Ethanol was detected with a differential refractometer (Merck RI-71), where the quantification limit was 5 μg/ml. The retention time for ethanol was 2.5 min.

Data Analysis

The permeability coefficients of E_2 in membrane and membrane with adjacent adhesive were calculated using Eq. 1:

$$P = \frac{J_{SS}}{C_D} \tag{1}$$

where P is the permeability coefficient, J_{SS} is the steady state flux of E_2 through the barrier layer and C_D is the drug concentration in the donor.

The diffusion coefficient of E_2 in the EVA membrane for the symmetric case was calculated using Eq. 2:

$$D = \frac{P \times h}{K} \tag{2}$$

where h is the effective thickness of the EVA membrane (50 μm, confirmed by microscopy) and K is the membrane/donor solution partition coefficient.

Permeability of the adhesive layer in the symmetric case was calculated using Eq. 3:

$$\frac{1}{P_A} = \frac{1}{P_{MA}} - \frac{1}{P_M} \quad (3)$$

where P_A, P_{MA} and P_M are the permeability coefficients of the adhesive, composite of membrane and adhesive and the single membrane, respectively.

RESULTS AND DISCUSSION

Symmetric Case

The influence of the EVA membrane and the adhesive layer on E_2 flux from defined ethanol/PBS co-solvent systems was determined. These permeation studies were conducted using saturated solutions to ensure that the permeant is at its maximum chemical potential. To further simplify the analysis, aqueous ethanol solutions were present in the donor and the receptor compartment at equal concentrations (symmetric case) to avoid ethanol gradients across membrane and adhesive layer.

The steady state flux rates of E_2 across the uncoated EVA membrane are shown in Fig. 1. The E_2 flux increased from 0.099 ± 0.014 $\mu g \times cm^{-2} \times h^{-1}$ from PBS up to 2.680 ± 0.273 $\mu g \times cm^{-2} \times h^{-1}$ in 96% (v/v) ethanol. If there were no interactions between the solvent and the membrane, the flux of E_2 from each saturated solvent system should be constant (21). However, a 27-fold increase in drug flux was seen, implying considerable interactions between solvent and membrane, which were characterized by the determination of physicochemical parameters relevant for E_2 diffusion through EVA membranes.

The solubility of E_2 in defined ethanol/PBS mixtures and the membrane/donor solution partition coefficient as a function of ethanol concentration are summarized in Fig. 2. The solubility of E_2, which is in accordance with other studies (13), strongly increased with increasing ethanol concentrations. This behavior leads to a decreasing partition coefficient, which represents the proportion of E_2 uptake by the membrane and solubility of drug in the donor. In contrast to the assumption of an inert membrane,

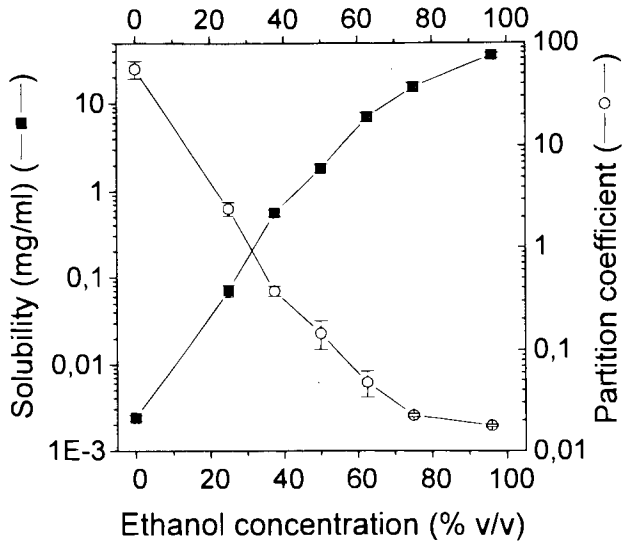

Fig. 2. Influence of ethanol concentration on E_2 solubility (-■-; n ≥ 6) and the membrane/donor solution partition coefficient (-○-; n ≥ 3).

the uptake of E_2 by the membrane (Fig. 3) increased nearly linearly with increasing ethanol concentrations. Furthermore, from Fig. 3 it is obvious that the E_2 uptake is directly proportional to the sorption of ethanol into the membrane from ethanol/PBS mixtures.

Therefore, it could be concluded that increasing amounts of ethanol embedded in the membrane are responsible for an enhanced solubility of E_2 in the polymer. A markedly enhanced uptake of E_2 and ethanol is observed at ethanol concentrations >75% (v/v), which is reflected in the non-proportional rise in E_2 flux across the EVA membrane at these higher ethanol concentrations.

Since the thickness of the membrane (h) was not significantly altered in the presence of ethanol (data not shown), the apparent diffusion coefficients of E_2 in the membrane could

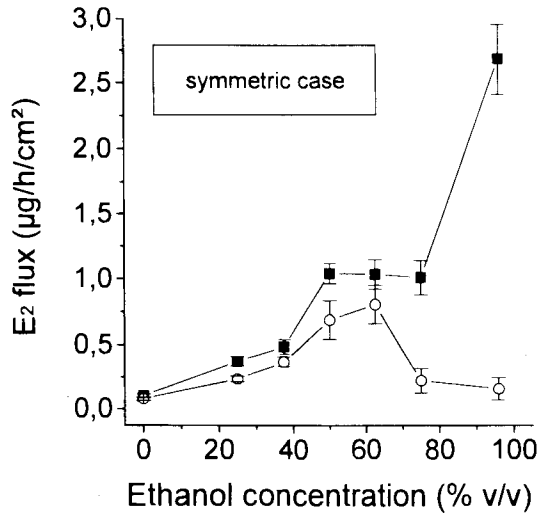

Fig. 1. Influence of ethanol concentration on E_2 flux across EVA membrane (-■-) and EVA membrane coated with adhesive (-○-) in the symmetric configuration. Each data point represents the mean and standard deviation of at least three determinations.

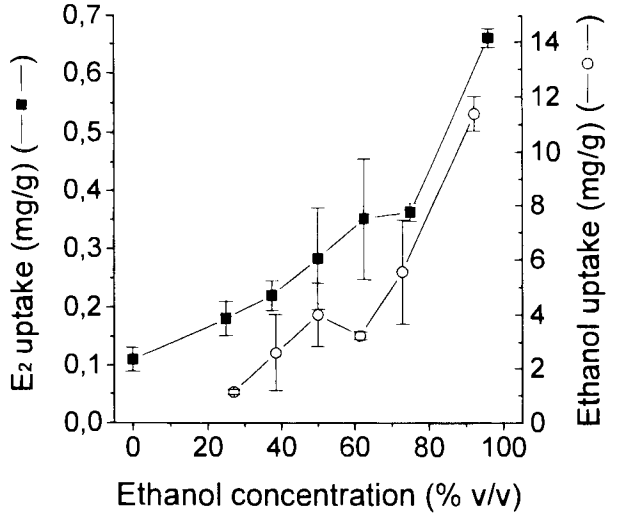

Fig. 3. The effect of different ethanol concentrations on the uptake of E_2 from saturated ethanol/PBS co-solvent systems (-■-) and the uptake of ethanol (-○-) by the EVA membrane. Each data point represents the mean and standard deviation of at least three determinations.

be calculated by Eq. 2 using the experimentally determined permeabilities and partition coefficients. From Fig. 4, where the apparent diffusion coefficients are presented as a function of ethanol concentrations, it is obvious that the diffusivity of the membrane increased with increasing ethanol concentrations.

An interaction between ethanol and the polymeric structure seems to be a likely explanation. To evaluate reversibility of the effect of ethanol on the polymer, discs of the membrane were preincubated with 96% (v/v) ethanol for 24 hours, washed and used for permeation studies in side-by-side diffusion cells. This pretreatment had no influence on the experimentally determined permeabilities from defined ethanol/PBS mixtures (data not shown). Therefore, the effect of ethanol was found to be completely reversible and not due to dissolution or extraction of polymer components from the EVA membrane. These findings suggest that ethanol acts by a reversible plasticizing effect leading to reduced barrier properties of this EVA membrane.

For an EVA membrane containing 12% vinyl acetate, an enhanced permeability for nitroglycerin and ethanol with increasing ethanol concentrations was directly attributed to such a plasticizing effect (22). Here, in case of the 9% EVA membrane, it was shown that the increasing E_2 flux across the EVA membrane with increasing ethanol concentrations is due to a larger uptake of E_2 by the membrane and the increasing diffusivity of the polymer. Both parameters are directly related to an increasing uptake of ethanol by the membrane.

When the 9% EVA membrane was coated with adhesive, a different profile for the E_2 flux rate versus ethanol concentration curve was observed. The E_2 flux across membrane and adhesive presented in Fig. 1 increased with increasing ethanol concentration up to a maximum flux (J_{SS}) of 0.803 ± 0.145 $\mu g \times cm^{-2} \times h^{-1}$ at 62.5% (v/v) ethanol, decreasing then with further increase in the volume fraction of ethanol. This suggests that the adhesive layer used for the MCRS has an important influence on the overall drug flux.

In order to clarify the extent of both barriers involved in E_2 flux control, the experimentally determined permeabilities of the EVA membrane and the composite of EVA membrane and adhesive layer, as well the calculated permeability for E_2 of the adhesive layer (Eq. 3), were compared (Fig. 5). At lower ethanol concentrations the EVA membrane represents the rate limiting barrier. When the ethanol content exceeds 62.5% (v/v), the permeability of the adhesive layer decreases dramatically, while the permeability of the membrane levels off, leading to a 15-fold lower permeability of the adhesive layer compared to the membrane at 96% (v/v) ethanol.

Therefore, the adhesive layer consisting of a mixture of polyisobutenes and light mineral oil is the rate controlling barrier for E_2 release from the MCRS, when the ethanol content exceeds 70% (v/v) in the symmetric case.

The experiments discussed so far were conducted with the membrane and/or adhesive layer. The question of the rate limiting barrier in a sandwich of EVA membrane, adhesive and human skin is of practical importance. Experimental data for E_2 permeabilities of human skin in dependence on ethanol concentrations in the symmetric case were taken from the literature and were compared to obtained permeabilities of the EVA membrane coated with adhesive (Table 1). For ethanol concen-

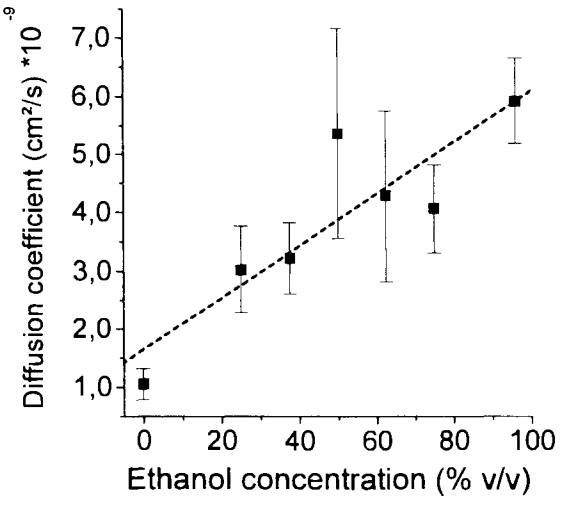

Fig. 4. Calculated diffusion coefficients of E_2 through 9% EVA membrane from ethanol/PBS mixtures.

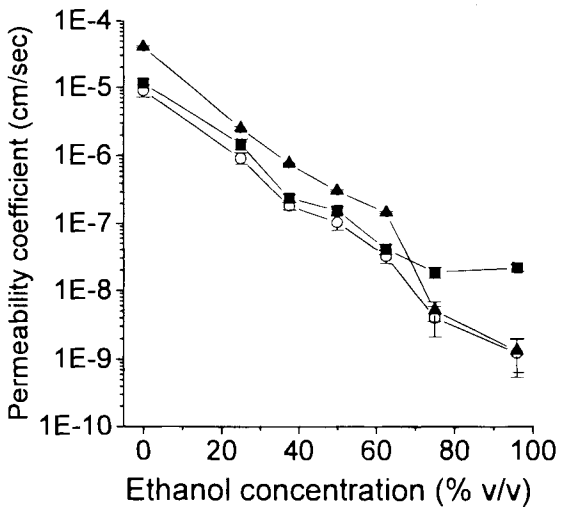

Fig. 5. The effect of different ethanol concentrations in the symmetric configuration on the experimentally determined permeability coefficients of E_2 across EVA membrane (-■-) and EVA membrane coated with adhesive (-○-) as well as calculated permeability of E_2 across the adhesive layer (-▲-).

Table 1. Comparison of E_2 Permeabilities for Human Skin (Taken from the Literature) and for the EVA Membrane Coated with Adhesive Layer in the Symmetric Case

Ethanol concentration (% v/v)	Human skin[a] P_{HS} (cm × sec^{-1})	EVA membrane + adhesive P_{MA} (cm × sec^{-1})
25	$1.76 \pm 0.93 \times 10^{-6}$[b]	$9.23 \pm 1.71 \times 10^{-7}$
50	$3.64 \pm 0.12 \times 10^{-7}$[b]	$1.03 \pm 0.25 \times 10^{-8}$
75	$2.78 \pm 0.56 \times 10^{-7}$[c]	$3.94 \pm 1.84 \times 10^{-9}$
95	$5.28 \pm 1.67 \times 10^{-7}$[d]	$1.23 \pm 0.69 \times 10^{-9}$

[a] Data were taken from the literature. All experiments were conducted in side-by-side diffusion cells in the symmetric configuration using saturated solutions of E_2.
[b] Permeabilities were calculated from E_2 fluxes displayed in Ref. 12 using the solubilities determined in our study.
[c] Data were taken from Ref. 23.
[d] Data were taken from Ref. 24.

trations from 25 to 95% (v/v) the permeability of human skin is significantly higher than the permeability of membrane and adhesive, indicating that the patch, and not the skin, limits the E_2 flux in this symmetric case.

Asymmetric Case

The asymmetric configuration represents the practically significant situation. In this configuration the *in vivo* use of MCRS is simulated, where drug and enhancer are released from an ethanolic reservoir across the stratum corneum into an aqueous medium, the human body. However, in this case, steady state ethanol gradients across the diffusion barrier may occur due to the simultaneous transport of E_2 and ethanol (25), leading to considerably more difficult data analysis.

In Fig. 6, E_2 flux rates across single EVA membrane and membrane coated with adhesive for the asymmetric case in dependence on donor ethanol concentrations are shown. For both curves a profile comparable to the symmetric case could be observed, although the absolute flux rates are somewhat lower in this case.

As already shown in the symmetric case, both curves yield a similar pattern up to 62.5% (v/v) ethanol, but differed significantly at higher alcohol concentrations. The permeation across the EVA membrane steadily rose with increasing ethanol concentrations in the donor. Here a 7.5 fold increase could be observed when the E_2 flux rates from pure PBS and from ethanol of 96% (v/v) were compared.

The E_2 flux across EVA membrane coated with adhesive increased up to a maximum value of 0.366 ± 0.024 $\mu g \times cm^{-2} \times h^{-1}$ at 62.5% ethanol followed by a decrease with increasing ethanol content in the donor solution, indicating that the adhesive layer again represents the rate controlling barrier at donor ethanol concentrations ≥70% (v/v).

The ethanol fluxes determined simultaneously are presented as a function of donor ethanol content in Fig. 7. As seen for E_2 fluxes, the permeation of ethanol across the membrane exhibited a nearly linear dependence on donor ethanol concentrations from 0.477 ± 0.356 $mg \times cm^{-2} \times h^{-1}$ at 25% ethanol up to 1.866 ± 0.267 $mg \times cm^{-2} \times h^{-1}$ at 96% ethanol. When membrane and adhesive layer were employed, a maximum ethanol flux of 1.263 ± 0.308 $mg \times cm^{-2} \times h^{-1}$ at 62.5% (v/v) ethanol followed by a decrease at higher donor ethanol contents could he observed.

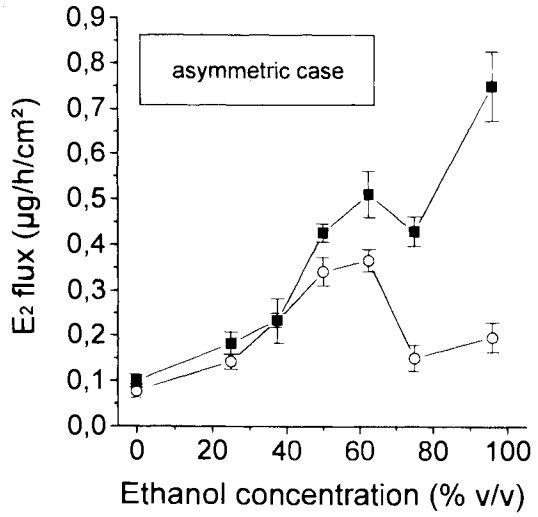

Fig. 6. Influence of donor ethanol concentration on E_2 flux across EVA membrane (-■-) and EVA membrane coated with adhesive (-○-) in the asymmetric configuration. Each data point represents the mean and standard deviation of at least three determinations.

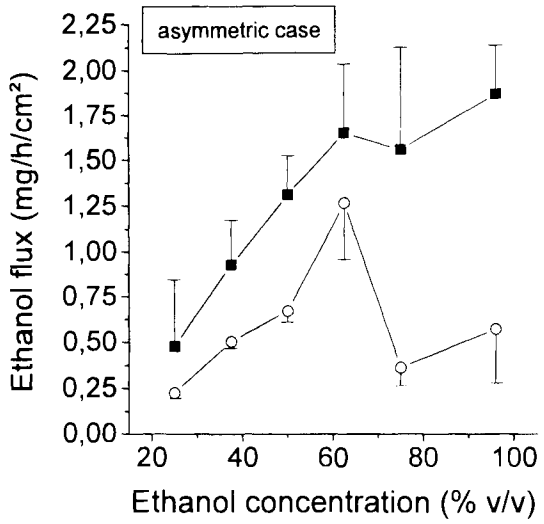

Fig. 7. Influence of donor ethanol concentration on ethanol flux across EVA membrane (-■-) and EVA membrane coated with adhesive (-○-) in the asymmetric configuration. Each data point represents the mean and standard deviation of three determinations.

Since similar flux rate versus donor ethanol concentration curve profiles for E_2 and ethanol could be observed, a nearly linear relationship between E_2 and ethanol fluxes could be assumed. A similar linear dependence was previously shown for permeation of E_2 (12,15) as well as permeation of nitroglycerin (18) across human skin, when the drug was dissolved in ethanol/water mixtures. It was argued that the drug should be co-transported with the solvent ethanol (15,18). From our investigations, it seems that this linear relationship between drug and ethanol fluxes is also valid for artificial polymeric diffusion barriers as the 9% EVA membrane as well as an adhesive consisting of polyisobutenes.

The experiments conducted here can be used to interpret *in vitro* experiments, where the transdermal E_2 flux rate across a sandwich of MCRS membrane, adhesive and excised human skin was determined in the asymmetric configuration (8). In this study an E_2 flux rate maximizing at 62.5% (v/v) ethanol and then decreasing at higher donor ethanol contents was observed. This profile indicates, that the transdermal E_2 flux is strictly dependent on ethanol concentrations in the reservoir of the patch. At ethanol contents ≥70% (v/v) the adhesive layer of the MCRS controls the release of E_2 from the patch into the body, and not the EVA membrane, as claimed by the manufacturer. If the ethanol content in the reservoir changes, which may occur due to the release of ethanol during the application (11), a non constant transdermal flux rate results. Such a fluctuating input rate was recently observed *in vitro* and *in vivo* for the investigated MCRS delivering E_2 (8).

Investigations presented here, where the release kinetic of the MCRS was directly related to the composition of the patch may be helpful for designing improved transdermal reservoir systems. It

should be possible to optimize MCRS by adjusting the ethanol content in the reservoir as well as by the selection of materials to get sufficiently high and stable transdermal input rates.

CONCLUSIONS

In vitro investigations in the kinetics of a commercially available membrane controlled reservoir system (MCRS) delivering β-estradiol (E_2) were conducted to elucidate mechanisms responsible for drug flux control. A comparison of our data to investigations conducted with human skin revealed that the MCRS design controls the transdermal E_2 input rate.

Furthermore, it was shown that the release of E_2 from the TDS is strictly dependent on the ethanol content in the reservoir of the patch: When a MCRS is applied to the skin *in vivo*, the adhesive layer consisting of a mixture of polyisobutenes and light mineral oil represents the rate controlling barrier, and not the EVA membrane, as claimed by the manufacturer. Since ethanol is released from the patch during the application, the reservoir ethanol concentration steadily decreases leading to changes in E_2 flux control. At ethanol contents below ca. 70% (v/v) the E_2 flux is mainly controlled by the EVA membrane. With further decrease in the ethanol content the E_2 flux decreases due to a decreasing uptake of E_2 by the EVA membrane as well as a decreasing diffusivity of the polymer, both parameters directly correlated to the ethanol uptake by the membrane.

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