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In vitro skin permeation of estradiol from various proniosome formulations

Jia-You Fang [a,b], Song-Yih Yu [a], Pao-Chu Wu [a], Yaw-Bin Huang [a], Yi-Hung Tsai [a,*]

[a] *School of Pharmacy, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, Kaohsiung 807, Taiwan*
[b] *Graduate Institute of Pharmaceutical Sciences, Taipei Medical University, Taipei 110, Taiwan*

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Abstract

The skin permeation of estradiol from various proniosome gel formulations across excised rat skin was investigated in vitro. The encapsulation efficiency and size of niosomal vesicles formed from proniosomes upon hydration were also characterized. The encapsulation (%) of proniosomes with Span surfactants showed a very high value of ≒100%. Proniosomes with Span 40 and Span 60 increased the permeation of estradiol across skin. Both penetration enhancer effect of non-ionic surfactant and vesicle-skin interaction may contribute to the mechanisms for proniosomes to enhance estradiol permeation. Niosome suspension (diluted proniosomal formulations) and proniosome gel showed different behavior in modulating transdermal delivery of estradiol across skin. Presence or absence of cholesterol in the lipid bilayers of vesicles did not reveal difference in encapsulation and permeation of the associated estradiol. The types and contents of non-ionic surfactant in proniosomes are important factors affecting the efficiency of transdermal estradiol delivery.

Keywords: Estradiol; Permeation; Proniosomes; Niosomes; Non-ionic surfactants

1. Introduction

Niosomes are unilamellar or multilamellar vesicles formed from synthetic non-ionic surfactants, offering an alternative to liposomes as drug carriers (Uchegbu and Vyas, 1998). An increasing number of non-ionic surfactants have been found to form vesicles, capable of entrapping hydrophilic and hydrophobic solutes (Yoshioka et al., 1994). Niosomes are important from a technical viewpoint as they possess greater stability and avoid some disadvantages associated with liposomes such as variable purity of phospholipids and high cost (Vora et al., 1998). Another advantage of niosomes is the development of a simple practical method for the routine and large-scale production without the use of pharmaceutically unacceptable solvents. Proniosomes (or proliposomes) offer a versatile vesicle delivery concept with potential for delivery drugs via transdermal

* Corresponding author. Tel.: +886 2 2707 9823; fax: +886 2 2707 4804.
E-mail address: fajy@ms9.tisnet.net.tw (Y.-H. Tsai).

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route (Vora et al., 1998; Huang et al., 1997). This would be possible if proniosomes form niosomes upon hydration with water from skin following topical application under occlusive conditions. The aim of this study is to examine the feasibility of proniosomes as a transdermal drug delivery system of estradiol.

Estradiol was selected as a model lipophilic drug in this study because of its potential application in the case of hormonal insufficiencies (Balfour and Heel, 1990). To optimize and evaluate various estradiol formulations, proniosomes were prepared by using two types of non-ionic surfactant including Span and Tween. The preparation method used in this study was based on the simple idea that mixtures of phospholipids, non-ionic surfactants, ethanol and water can be used to form a concentrated proniosome gel which can be converted to a stable niosome suspension by hydration with excess aqueous phase (Perrett et al., 1991). To characterize physicochemical properties of this dispersion such as vesicle size and drug encapsulation percentage was also assessed for estradiol proniosomes. In the in vitro permeation study, excised rat skin mounted in Franz (vertical) diffusion cell under occlusive condition was used. In order to verify the possible mechanism of estradiol permeating across skin from proniosomes, a series of permeation studies were also examined in this present study: (i) estradiol release rate across cellulose membrane, (ii) estradiol permeation across skin pretreated with surfactants and phospholipids, and (iii) estradiol permeation from reconstituted niosome suspension.

2. Materials and methods

2.1. Materials

Span 40, Span 60, Span 85 and 17β-estradiol were obtained from Tokyo Kasei Co. (Japan). L-α-lecithin (from fresh egg york, ≈99%) and cholesterol were purchased from Sigma Co. (USA). Tween 20, Tween 40 and Tween 80 were supplied from Merck Co. (Germany). The artificial cellulose membrane (Spectra/por® 2) was obtained from Spectrum Medical Ind. Co. (USA). All other chemicals and solvents were of analytical grade.

2.2. Preparation of proniosomes

Proniosomes were prepared by a method modified from Perrett et al., 1991. Five mg of estradiol with surfactant, lecithin and cholesterol were mixed with 125 μl absolute ethanol in a wide mouth glass tube. The composition of additives are listed in Table 1. Then the open end of the glass tube was covered with a lid and warmed in a water bath at 65 ± 3°C for 5 min. A 80 μl; pH 7.4 phosphate buffer was added and still warmed on the water bath for about 2 min till the clear solution was observed. The mixture was allowed to cool down at room temperature till the dispersion was converted to proniosomal gel.

Table 1
Composition of various proniosomal formulations (mg)

Proniosome code	Estradiol	Span 40	Span 60	Span 85	Tween 20	Tween 60	Tween 80	Lecithin	Cholesterol
S40	5	90	–	–	–	–	–	90	10
S60	5	–	90	–	–	–	–	90	10
S85	5	–	–	90	–	–	–	90	10
T20	5	–	–	–	90	–	–	90	10
T60	5	–	–	–	–	90	–	90	10
T80	5	–	–	–	–	–	90	90	10
S1L2	5	60	–	–	–	–	–	120	10
S2L1	5	120	–	–	–	–	–	60	10
Non-CH	5	90	–	–	–	–	–	90	–

2.3. Estradiol encapsulation percentage determination

The proniosome gel in the glass tube was reconstituted with 10 ml; pH 7.4 phosphate buffer. The aqueous suspension was sonicated in a sonicator bath. The estradiol-containing niosomes were separated from untrapped drug by centrifuging at 25 000 rpm at 20°C for 30 min. The supernatant was taken and diluted with methanol. The estradiol concentration in the resulting solution was assayed by HPLC method. The percentage of drug encapsulation was calculated by the following equation:

$$\text{EP}\ (\%) = [(C_t - C_f)/C_t] \times 100,$$

where C_t is the concentration of total estradiol, and C_f is the concentration of free estradiol.

2.4. Vesicle size analysis

Light scattering measurements were performed with a Coulter submicron particle-size analyzer (Model N4MD, Coulter, USA). The proniosome gel in the glass tube was diluted with 10 ml; pH 7.4 phosphate buffer to determine the vesicle size. The instrumental settings were temperature, 20°C; viscosity, 0.01 poise; refractive index, 1.333; reference angle, 90°C; run time, 200 s; and range, 0–3000 nm.

2.5. In vitro permeation study

The permeation of estradiol from proniosome formulations was determined by using Franz (vertical) diffusion cell. The Wistar rat (7–9 weeks old) skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment. The top of the diffusion cell was covered with paraffin paper. The donor compartment was filled with the proniosome formulation as shown in Table 1. A 20 ml aliquot of 40%:60% (v/v) ethanol/pH 7.4 phosphate buffer was used as receptor medium to maintain a sink condition. The available diffusion area of cell was 2.54 cm^2. The receptor compartment was maintained at 37°C and stirred by a magnetic bar at 600 rpm. At appropriate intervals, 200 μl aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution. The samples were analyzed by HPLC method. The laboratory temperature was kept at 25 ± 1°C and the relative humidity was about 60%.

The permeation of estradiol from niosome suspensions (diluted proniosome formulations) was determined by the side-by-side diffusion cell. The niosome dispersion diluted from proniosome gel with 10 ml; pH 7.4 phosphate buffer was used as the donor phase. A 8 ml aliquot of 40%:60% (v/v) ethanol/pH 7.4 phosphate buffer was used as receptor medium. The available diffusion area of cell was 0.785 cm^2. The other procedures were the same as those of Franz diffusion cell experiment.

2.6. HPLC analytical method

The estradiol content of the various samples was analyzed by an HPLC system consisting of a Jasco Model 880-PU HPLC pump, a Jasco Model 855-AS sample processor and a Jasco Model 875-UV detector. A 12.5 cm long, 4.0 mm inner diameter stainless steel column with RP-18 column (Merck) was used. The estradiol sample was mixed with a suitable amount of clobetasol 17-propionate as the internal standard. The mobile phase consisting of 35%; 0.05% acetic acid in water and 65% methanol was used at a flow rate of 1.0 ml/min. The UV detector was set at a wavelength of 280 nm. The retention time of estradiol and internal standard was found to be 5.5 and 10.2 min, respectively.

3. Results and discussion

3.1. Encapsulation efficiency and vesicle size of various proniosome formulations

Proniosomes prepared with non-ionic surfactants of alkyl ester including Span (sorbitan esters) and Tween (polyoxythylene sorbitan esters) were utilized to determine the encapsulation of associated estradiol and vesicle size. As shown in Table 2, encapsulation efficiency of niosomes formed from S40, S60 and S85 proniosome gel

Table 2
Characterization of proniosomal formulations by encapsulation and vesicle size after dilution with buffer[a]

Proniosome code	Encapsulation (%)	Vesicle size (nm)
S40	97.76 ± 0.19	978
S60	97.26 ± 0.50	870
S85	97.18 ± 0.82	1116
T20	84.87 ± 1.18	644
T60	63.15 ± 3.96	347
T80	55.37 ± 4.07	447
S1L2	98.03 ± 0.23	841
S2L1	97.99 ± 0.21	1001
Non-CH	97.87 ± 0.25	1245

[a] Each value represents the mean ± SD ($n = 3$).

exhibits a very high value of ≒100%. Highly lipophilic drugs like estradiol are intercalated almost completely within the lipid bilayer of liposomes and niosomes (Gulati et al., 1998). This result was consistent with the entrapment efficiency of levonorgestrel in proniosomes incorporated with Span 40 (Vora et al., 1998). Most of the surfactants used to make non-ionic surfactant vesicles have a low aqueous solubility. However, freely soluble non-ionic surfactants such as Tween can form the micelles on hydration in the presence of cholesterol (Uchegbu and Vyas, 1998). The encapsulation of T20, T60 and T80 formulations in our study also showed estradiol can be entrapped into niosomes composed of Tween; however, the encapsulation efficiency was relatively low as compared to those composed of Span (Table 2). This is because the vesicles can be successfully formed by Tween only in the presence of cholesterol in excess of 33.33 mol% (Uchegbu and Florence, 1995). As the cholesterol content of the formulation decreased, the encapsulation of drug also decreased.

The mean vesicle size of niosomes formed from estradiol proniosome formulations is given in Table 2. The differences of vesicle size among all niosomes with Span were not great. On the other hand, the size distribution of niosomes with Tween was significantly lower than that with Span surfactant.

3.2. In vitro permeation of estradiol from proniosomes

Fig. 1 shows the flux of estradiol permeated from proniosomes to the in vitro receptor compartment. The formulation of control group was estradiol suspended in the same solvent of proniosome formulation. The proniosome formulations selected here were the formulations with high encapsulation efficiency for estradiol in Table 2. The flux of estradiol from S40 and S60 was significantly higher (t-test, $P < 0.05$) than that of control group. However, no significant difference (t-test, $P > 0.05$) was observed in skin permeation profile of S85 and T20 formulations and control group. Proniosomes should be hydrated to form niosomal vesicles before estradiol released and permeated across the skin. As observed in the in vitro permeation experiment, the cumulative amount of estradiol in receptor was detected in the first sampling time (0.5 h) for all proniosome formulations. This indicated that all the procedures of estradiol proniosome permeation including water penetration from the receptor compartment to skin, conversion of proniosomes to niosomal vesicles, estradiol release from niosomes and permeation of estradiol across skin occur very rapidly.

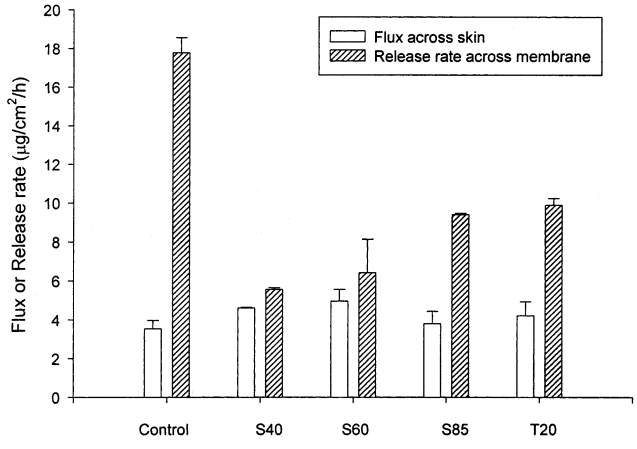

Fig. 1. The flux and release rate of estradiol from various proniosomal formulations across skin and cellulose membrane. Each value represents the mean ± SD ($n = 3$).

Several mechanisms can be used to explain the ability of niosomes to modulate drug transfer across skin (Uchegbu and Vyas, 1998; Vora et al., 1998; Touitou et al., 1994; Schreier and Bouwstra, 1994), including (i) adsorption and fusion of niosomes onto the surface of skin leading to a high thermodynamic activity gradient of drug at the interface, which is the driving force for permeation of lipophilic drugs, (ii) the effect of vesicles as the penetration enhancers to reduce the barrier properties of stratum corneum, and (iii) the lipid bilayers of niosomes as rate-limiting membrane barrier for drugs. In order to verify the predominant driving force for estradiol proniosomes permeating across skin, some efforts were made to clarify these mechanisms. The release rate of estradiol across cellulose membrane is presented in Fig. 1. The release rate of estradiol was significantly higher than its flux across skin, indicating the barrier properties of skin for estradiol. The trends of estradiol permeation from various formulations across rat skin and cellulose membrane were quite different. The control group showed the highest release rate of estradiol among all formulations. However, the release of estradiol from proniosomes was significantly retarded (t-test, $P < 0.05$). This can be explained by the fact that the mean pore size of the artificial membrane used in this study (molecular weight cut-off: 12 000–14 000) was less than 10 nm and only very small vesicles could diffuse intact across this membrane.

The release rate of estradiol across membrane from S85 formulation was significantly higher (t-test, $P < 0.05$) than those from S40 and S60 formulations, which was opposite to the result of estradiol permeation across skin. This may be attributed to the fact that molecules of Span 40 and Span 60 are in the ordered gel state at the in vitro permeation condition of 25°C. However, Span 85 is in the disordered liquid-crystalline state in the same condition (Yoshioka et al., 1994). The disorder of bilayer fluidity may result in the enhancement of bilayer permeability to solutes and hence the release rate of solutes. Since there was a great discrepancy between the permeation profiles of estradiol proniosomal formulations across skin and cellulose membrane, interaction between skin and proniosomes itself may be an important contribution for the improvement of transdermal estradiol delivery.

One of the possible reasons for niosomes to enhance the permeability of drugs is structure modification of stratum corneum. It has been reported that the intercellular lipid barrier in stratum corneum would be dramatically changed to be looser and more permeable by treatment with liposomes and niosomes (Schreier and Bouwstra, 1994; Coderch et al., 1996; Ogiso et al., 1996). Both phospholipids and non-ionic surfactants in proniosomes can act as penetration enhancers, which are useful for increasing the permeation of many drugs (Sarpotdar and Zatz, 1986; Nishihata et al., 1987; Yokomizo, 1996; Kirjavainen et al., 1999). To investigate the possibility of enhancing potential for phospholipids and non-ionic surfactants to increase estradiol permeation, the skin was pretreated by solutions of lecithin, Span and Tween for 12 h with the same concentration in the proniosome formulation, following administrating free estradiol suspension. Control experiment was performed under identical conditions without enhancers in the pretreatment solution. As shown in Table 3, the permeation of estradiol was slightly increased after pretreatment by solution without enhancers, although there was no significant difference (t-test, $P > 0.05$) as compared to the permeation of control group without any pretreatment. After comparing the pretreatment of enhancer with 90 mg amount, significant enhancement (t-test, $P < 0.05$) of estradiol permeation was only observed after pretreatment of Span 60. This effect may contribute to the highest estradiol flux from S60 proniosomal formulation across skin. The pretreatment of lecithin did not cause any enhancing effect possibly due to the insufficiency of lecithin amount since a high concentration of phospholipid molecules in vehicles in contact with skin surface is needed to strongly disrupt the lipid bilayers of stratum corneum (Yokomizo, 1996).

Besides the biological membranes, surfactants may affect the permeability of a solute through skin by interacting with the solute (Sarpotdar and Zatz, 1986; Tan et al., 1993). Fig. 2 depicts the permeation profile of estradiol participated with

Table 3
Penetration flux and enhancement ratio of estradiol across excised rat skin after pretreatment of surfactant and lecithin

Pretreatment compound	Pretreatment content (mg)	Flux ($\mu g/cm^2/h$)	ER[a]
Blank vehicle	–	4.78 ± 1.62	–
Span 40	60	4.62 ± 0.40	0.96
Span 40	90	6.47 ± 0.86	1.35
Span 40	120	7.18 ± 1.32	1.51
Span 60	90	10.14 ± 1.97	2.12
Span 85	90	5.08 ± 1.20	1.06
Tween 20	90	5.48 ± 1.11	1.15
Lecithin	90	4.56 ± 2.00	0.96

[a] ER = enhancement ratio = estradiol flux pretreated with surfactant or lecithin/estradiol flux pretreated with blank vehicle, each value represents the mean $\pm$ SD ($n = 3$).

the surfactant. The concentration of surfactants was the same with that in the proniosomal formulations. All the surfactants were in excess of their critical micelle concentrations. Surfactant addition generally reduced the skin permeation of estradiol (Fig. 2). It is reasonable since the micelles or complexes of non-ionic surfactant reduce thermodynamic activity and are too large to diffuse as such into skin (Sarpotdar and Zatz, 1986; Fang et al., 1998). This result indicates that surfactant itself may not increase the permeability of estradiol proniosomes.

Although the estradiol flux from S40 proniosomes was comparable to that from S60 proniosomes, the enhancing effect by Span 40 pretreatment with skin or Span 40 participation in donor vehicle was not evident (Table 3, Fig. 2). Accordingly mechanisms other than the disruption of stratum corneum lipid bilayers should be elucidated for S40 formulation. Most investigators agree that direct contact between vesicles and skin is essential for efficient delivery, although phospholipids apparently do not penetrate into deeper skin layers (Ganesan et al., 1984; Gesztes and Mezei, 1988; Schreier and Bouwstra, 1994). Lipophilic drugs like estradiol are intercalated within the bilayer structure of the lipids in niosomes. Theoretical analysis supports the concept that direct transfer of drug from vesicles to skin occurs only when the drug intercalated within bilayers (Weiner et al., 1989). If this mechanism is valid, vesicles are a more promising carrier for lipophilic drugs. Fusion of niosome vesicles of estradiol on the surface of skin has been demonstrated by electron microscopy in a recent report (Schreier and Bouwstra, 1994). In the previous study, estradiol encapsulated in niosomes results in much higher estradiol fluxes than when applied in a buffer solution. Consequently, for lipophilic estradiol in proniosomes, the experimental results and supportive theoretical analysis suggest direct transfer of drug from vesicles to the skin.

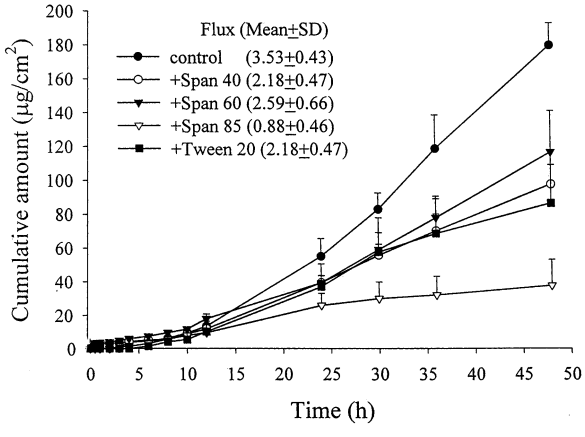

Fig. 2. Cumulative amount of estradiol detected in the receptor compartment versus time following transdermal absorption from various surfactant solutions across excised rat skin. *, significant difference to control group. Each value represents the mean $\pm$ SD ($n = 3$).

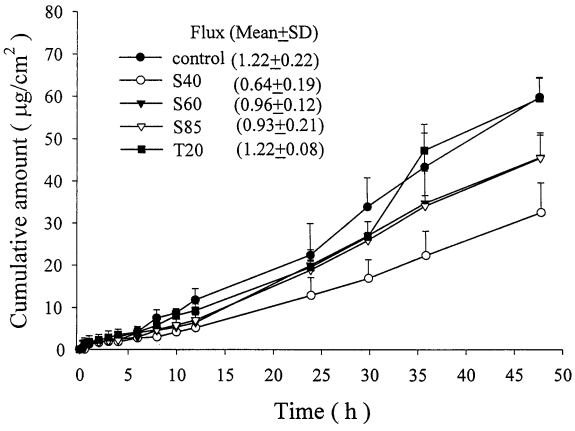

Fig. 3. Cumulative amount of estradiol detected in the receptor compartment versus time following transdermal absorption from various niosomal formulations (diluted proniosomal formulations) across excised rat skin. Each value represents the mean ± SD ($n = 3$).

3.3. In vitro permeation of estradiol from niosomes (diluted proniosome formulations)

The permeation of estradiol was also determined after dilution of proniosme gels by 10 ml; pH 7.4 buffer. This procedure was the same as that before detecting encapsulation efficiency and vesicle size of estradiol niosome vesicles. Fig. 3 shows the cumulative amounts of estradiol permeates from niosome formulations detected in the in vitro receptor compartment. The trend of estradiol flux from various niosome formulations (T20 > S60 ≧ S85 > S40) was quite different to that from proniosomal formulations (Fig. 2). This indicates that niosomes and proniosomes show different behaviors on influencing estradiol permeation across skin. Free estradiol passed through the skin with comparable facility to estradiol in niosomes, suggesting the lipid bilayers of niosomes are rate limiting membrane barriers for estradiol. The lower permeation of estradiol from niosomes is due to the significant dilution of proniosome formulations. The mechanisms by which proniosomes permeate across skin such as penetration enhancer effect and vesicle-skin interaction may be ignored in the status of niosome suspension since a high concentration of phospholipid and non-ionic surfactant is necessary to enhance the permeation of drugs from vesicles (Sarpotdar and Zatz, 1986; Yokomizo, 1996).

The estradiol flux from T20 niosomes was higher than that from niosomes incorporated with Span (Fig. 3). This may be due to the lower encapsulation of estradiol by T20 niosomes, hence the greater amount of free drug in formulations. Another explanation is that non-ionic surfactants with higher hydrophilicity like Tween appear to decrease stability of niosome suspension and increase leakage of free drug which can improve transdermal delivery of lipophilic molecules (Uchegbu and Vyas, 1998).

3.4. In vitro permeation of estradiol from proniosomes with various surfactant/lecithin ratios

Permeation of estradiol from S40 proniosome formulation with various surfactant/lecithin ratios was conducted and the result is shown in Fig. 4. Although S40, S1L2 and S2L1 formulations showed similar behavior on the encapsulation efficiency and vesicle size, a discrepancy was observed in the estradiol permeation. The flux of estradiol from S40 and S2L1 was significantly higher (t-test, $P < 0.05$) than that from S1L2, indicating a sufficient amount of non-ionic surfactant is needed to yield efficient estradiol delivery by the transdermal route. As shown in Table 3,

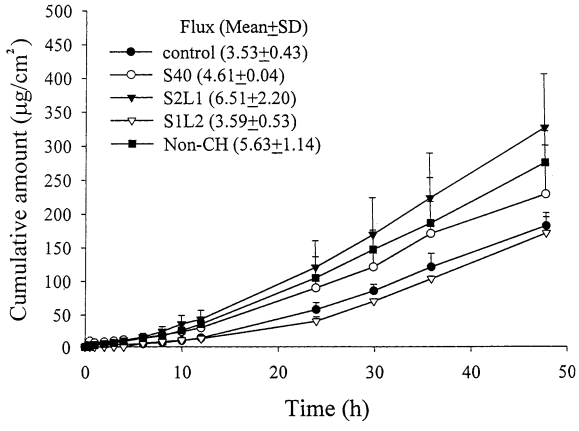

Fig. 4. Cumulative amount of estradiol detected in the receptor compartment versus time following transdermal absorption from various proniosomal formulations across excised rat skin. Each value represents the mean ± SD ($n = 3$).

the flux of estradiol increases following the increase of pretreatment concentration of Span 40. Accordingly, the penetration enhancer effect of surfactant may be an important contribution for estradiol permeates from proniosomes.

Previous study suggests that reducing the cholesterol content of niosomes increases the transdermal delivery of estradiol (Vanhal et al., 1996). This phenomenon cannot be observed in our result since there was no significant difference (t-test, $P > 0.05$) between the estradiol flux from S40 and non-cholesterol proniosomes (Fig. 4). This may also indicate the different mechanisms of estradiol transport across skin from niosomes and proniosomes. Further investigation is needed and in progress to explore the role of cholesterol in proniosomes.

4. Conclusions

The in vitro permeation of estradiol from proniosomes with various types and contents of non-ionic surfactant was evaluated in this study. The essential feature of proniosomes is their ability to rearrange on dilution to form a stable niosome suspension. Estradiol included in proniosomes was entrapped within the lipid bilayers formed by this technique with very high efficiency. Estradiol from proniosomes seems to pass through the skin with comparable facility to free drug. The experimental result and supportive theoretical analysis suggest either direct transfer of drug from vesicles to the skin or the penetration enhancer effect by non-ionic surfactant may contribute to the mechanism of estradiol permeation from proniosomal formulations. On the other hand, the enhancing permeation of estradiol from niosomes (diluted proniosome formulations) was not observed. This indicates that only proniosome gel not niosome suspension appears to efficiently deliver estradiol by the transdermal route in this study. A significant amount of non-ionic surfactant (Span 40) in the proniosomal formulation was needed to enhance estradiol permeation across skin. No significant difference was observed comparing the encapsulation efficiency and skin permeation of estradiol from proniosomes containing cholesterol to that determined from proniosomes without cholesterol. These findings suggest that inclusion of surfactants and lecithin in vesicles may play a more important role than inclusion of cholesterol in determining estradiol permeation. Proniosomes may become a useful dosage form for estradiol, specifically due to their simple, scaling-up production procedure and ability to modulate drug transfer across skin.

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