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Skin delivery of oestradiol from lipid vesicles: importance of liposome structure

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Abstract

The aim of this study was to investigate the importance of liposome structure in oestradiol skin delivery as a tool for understanding the delivery mechanism from lipid vesicles. Liposomes of phosphatidylcholine (PC) (I), PC, sodium cholate; 86:14 w/w (II), PC, Span 80; 86.7:13.3 w/w (III) and PC, oleic acid; 84:16 w/w (IV) with 1 mg/ml radiolabelled drug were prepared. Saturated radiolabelled oestradiol solutions containing the components of I-IV were separately prepared in 90% w/w propylene glycol in water. In addition, saturated solutions containing cholate, Span, oleic acid and ethanol at the same concentrations used in vesicles were formulated. Oestradiol permeation through human epidermis was studied. Formulations I-IV increased oestradiol flux by 8.6, 17, 17 and 13-fold when used as vesicles compared with control and by 2.9, 4.0, 4.7 and 6.9-fold when used in solution with drug. Testing individual components in solution, relative fluxes were 2.9, 0.87, 1.1, 2.9 and 1.1 for PC, cholate, Span, oleic acid and 7% ethanol, respectively. Accordingly, it is important to prepare phospholipids as vesicles for efficient oestradiol skin delivery even after inclusion of oleic acid. Penetration enhancement is not the main mechanism for improved flux. Liposome components in solution have additive effect with a possible synergism in some cases. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Skin delivery; Oestradiol; Lipid vesicles; Vesicular structure; Liposomes

1. Introduction

The potential use of lipid vesicles in topical delivery of drugs has been reported (Mezei and Gulasekharam, 1980, 1982). Since then, conflicting results have been recorded concerning the mode and mechanisms of action of liposomes as skin delivery systems. An effect whereby liposomes localised the drug in the stratum corneum, viable epidermis, and appendages, but reduced its systemic absorption, has been considered (Mezei and Gulasekharam, 1980, 1982). Depending on the type of liposomes, and/or the physicochemical properties of the drug as well as the additives, dermal and in some cases transdermal delivery has been shown (Mezei, 1992). Consequent on the

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nature of the drug, the possibility of a free drug mechanism, direct transfer between vesicles and skin or a combination of both procedures was postulated (Ganesan et al., 1984). Ultrastructural changes in the stratum corneum were seen after application of liposomes containing high concentrations of phosphatidylcholine (PC) (Hofland et al., 1995). Skin pretreatment with liposomes was reported to be valueless and it was concluded that liposomes must be applied concurrently with the drug or that the drug must be encapsulated into intact liposomes to achieve a positive effect (Du Plessis et al., 1994).

We have reported that lipid vesicles improved the skin delivery of oestradiol in vitro (El Maghraby et al., 1998). In mechanistic studies (El Maghraby et al., 1999) we investigated the effect of skin pretreatment with empty lipid vesicles on skin delivery of oestradiol. The results indicated an enhancing effect of drug-free PC liposomes but the enhancement ratio was less than that obtained after delivering the drug from the same vesicles. Other empty vesicles produced no significant effect on the epidermal permeability to oestradiol. These results suggested that lipids should be applied with the drug simultaneously but an important question remained; is it necessary to formulate lipid vesicles or can we simply prepare a solution of the lipids and drug in a suitable cosolvent?

In attempts to answer such questions, some authors emphasised the importance of vesicular structure by comparing skin delivery of cyclosporine from vesicles with that from emulsions having an emulsifier composition similar to the vesicles (Egbaria and Weiner, 1992). A similar conclusion was reported for niosomes (analogues of liposomes prepared from nonionic surfactants) simply from the negative results obtained for oestradiol after a pretreatment study compared with the positive data obtained after delivery from the same vesicles (Bouwstra and Junginger, 1995).

In the present study we report on the importance of vesicular structure in skin delivery of the lipophilic model drug, oestradiol, presented in liposomes. The investigation involved monitoring epidermal permeation of oestradiol after application of the drug in lipid vesicles. The results were then compared with those obtained after application of a saturated solution of oestradiol in 90% w/w propylene glycol/water containing the components of the individual formulations, i.e. in the absence of a liposomal structure. In addition, we monitored the effects of individual components of lipid vesicles to determine how those ingredients interact to influence the delivery of oestradiol from lipid solutions. The codes and the compositions of the tested formulations (vesicles and solutions) are presented in Table 1. The rationale for selection of the tested formulations is described in the Results and Discussion section.

Table 1
Codes and compositions of formulations (vesicles and solutions)[a]

Form and code	Composition
Vesicles-5% w/v	
I	Pure PC
II	PC, sodium cholate; 86:14 w/w
III	PC, span 80; 86.7:13.3 w/w
IV	PC, oleic acid; 86:14 w/w
Solutions	
CV	Saturated oestradiol in water (control for vesicles)
CS	Saturated oestradiol in 90% w/w PG/water (control for solutions)
S-I	Components of I dissolved in CS
S-II	Components of II dissolved in CS
S-III	Components of III dissolved in CS
S-IV	Components of IV dissolved in CS
S-Ch	Sodium cholate (7 mg/ml) dissolved in CS
S-Sp	Span 80 (6.65 mg/ml) dissolved in CS
S-Ol	Oleic acid (8 mg/ml) dissolved in CS
S-Eth	Ethanol (7% v/v) in CS

[a] PC, phosphatidylcholine; PG, propylene glycol.

2. Materials and methods

2.1. Materials

Oestradiol, [2,4,6,7-^{3}H(N)] was obtained from NEN Life Science Products. Phosphatidylcholine (PC) from soybeans (purity 99%), 17β-oestradiol (purity 98%), sodium cholate, sorbitan monooleate (Span 80), oleic acid (purity 95%),

1,2-propanediol (propylene glycol) and Sephadex G50 medium were purchased from Sigma Chemical (St Louis). All chemicals were used without further purification. Water was double distilled.

2.2. Preparation of lipid vesicles

Suspensions of 5% w/v lipid vesicles I-IV having compositions as in Table 1 were prepared by bath sonication (New, 1990) using a B12 FTZ bath sonicator and homogenised by manual extrusion. Briefly, the lipid components were dissolved in ethanol, then a radiolabelled solution of oestradiol in ethanol sufficient to produce a drug concentration of 1 mg/ml (25 μCi/ml) in the final preparation was added. The solvent was evaporated under a stream of nitrogen, before the last traces of the solvent were removed under vacuum overnight. The films were hydrated with either distilled water (pure PC liposomes, I) or 7% v/v ethanol in water (deformable formulations, II-III and oleic acid containing vesicles, IV) by vortexing for 15 min and the vesicles were left to swell for 2 h at room temperature before bath sonication for 30 min. The sonicated vesicles were homogenised by 10 times manual extrusion through a sandwich of 200 and 100 nm polycarbonate membrane.

2.3. Determination of entrapment efficiency

The entrapment efficiency was measured after separation of the non entrapped drug using the mini-column centrifugation method (Fry et al., 1978; New, 1990). The gel was prepared by leaving Sephadex G50 (10 g) to swell in distilled water (120 ml) at room temperature with occasional shaking for at least 5 h and was stored at 4°C. To prepare the mini-columns, Whatman GF/B filter pads were inserted in the bottom of the barrels of 2.5 cm^3 syringes which were then filled with gel. Excess water was removed by centrifugation at 3000 rpm for 3 min using a WIFUG Lab centrifuge (WIFUG, Bradford, UK), and 500 μl of liposomes was added dropwise to the center of the column, followed by centrifugation as before. To the mini-column, 625 μl of distilled water was added and centrifugation repeated. Depending on their type and sizes, liposomes can be recovered from the first or the first and second stages of centrifugation (New, 1990); here two stages were required to recover the vesicles. When saturated oestradiol solution was applied to the mini-column instead of the liposome suspensions, all of the free drug remained bound to the gel. This indicated the validity of the method as no free drug will be present after recovering the vesicles. The amount of drug entrapped in the vesicles was determined by liquid scintillation counting after correction for the dilution factor using the scintillation cocktail, OptiPhase 'HiSafe' 3, Fisher Chemicals, UK and a Tri Carb® Packard counter.

2.4. Determination of vesicle size

Diameters were determined using photon correlation spectroscopy employing a Zetasizer (Malvern Instruments, Malvern UK). Samples used distilled water filtered through 0.2 μm membranes to minimise interference from particulate matter. Vesicles were suitably diluted with filtered sodium chloride solution (1 mM in water) before sizing.

2.5. Preparation of saturated radiolabelled drug solutions

2.5.1. Saturated aqueous solution (CV)

Tritium labelled oestradiol (1 mCi) in ethanol was added to 5 ml ethanol containing 10 mg cold oestradiol. The solvent was evaporated under vacuum at ambient temperature. To the deposited crystals, 20 ml of distilled water was added and the mixture was heated to 60°C for 15 min. The suspension was left to equilibrate at 32°C whilst being agitated with a Teflon magnetic stirrer for 48 h. This resulted in a saturated radiolabelled oestradiol aqueous solution with excess radiolabelled crystals to maintain saturation.

2.5.2. Saturated solution in 90% propylene glycol (CS)

Propylene glycol in water (90% w/w) was used as an appropriate solvent to investigate how liposome components can influence the skin delivery of oestradiol if present in a simple mixed solution.

The goal was to research the importance of the structure of lipid vesicles in skin delivery of oestradiol. The reason for selection of this solvent is discussed in Section 3.

A saturated radiolabelled solution of oestradiol in 90% propylene glycol was prepared as before but the radioactive stock used produced 0.5 mCi/ml of saturated solution.

2.5.3. Saturated solution in 90% propylene glycol containing vesicles components

Saturated radiolabelled solutions of oestradiol in 90% propylene glycol were separately prepared containing the components of I-IV at the same concentrations used in liposomes, so as to yield the S series of solutions (Table 1). Saturated drug solutions containing sodium cholate, Span 80, oleic acid and ethanol in the same concentrations as used in the vesicles were also prepared. All solutions were maintained saturated as indicated by the presence of excess radiolabelled crystals of the drug.

2.6. Preparation of human epidermal membrane

Skin samples (Caucasian, postmortem, mid-line abdominal) were from 27 donors, 17 female, with an average age of 69.7 ± 12.1 years. The membranes were prepared by a heat separation technique (Kligman and Christophers, 1963). After removal of excess fat and connective tissue, the skin was soaked for 45 s in a water bath at 60°C. The epidermis was gently teased off by the tip of a gloved finger. Subsequently, a hydration protocol was designed to maintain the reported in vivo transepidermal hydration gradient (Warner et al., 1988) which has been claimed to generate the driving force for 'Transfersome' vesicular skin penetration (Cevc and Blume, 1992). The membranes were thus floated with the stratum corneum side up on 0.002% aqueous sodium azide and the upper surface was left open to the atmosphere for 24 h (open hydration protocol).

2.7. Permeation experiments

In vitro permeation studies used an automatic diffusion apparatus (Akhter et al., 1984) with flow through diffusion cells, diffusional areas of 0.126 cm^2. Sink conditions were maintained by pumping degassed receptor solution (0.002% w/v aqueous sodium azide) through the receptor compartment at a rate of 2 ml/h which allows for about 80 volume changes per h. Epidermal membranes were mounted with the stratum corneum side up and equilibrated for 12 h with receptor flowing and the donor compartment dry and open (non occluded hydration protocol). The system maintains the skin surface at 32°C.

The permeation studies involved a low dose design (20 μl open application). Saturated oestradiol in water (CV) was used as the control for lipid vesicles but a saturated solution in 90% propylene glycol (CS) was used as the control for studying the effects of lipids in solution form. For each study, the test and its control were performed on skin samples obtained from the same donors.

2.8. Data analysis

The use of alternative vehicles resulted in different permeation profiles for oestradiol after using the same dose (20 μl) and the same method of application. Lipid vesicles and their controls (saturated aqueous solution) dried within 1 h after application resulting in a cumulative amounts versus time plots (permeation profiles) tending towards a typical finite dose profile (Fig. 1). Solutions in 90% propylene glycol/water (test and control) did not dry throughout the experiment and thus provided a pseudo–steady state profile (Figs. 2 and 3).

The individual permeation profiles of the finite dose studies were used to calculate the rate plots. These rate plots were fitted by a polynomial (Fig. 1). The equations of the best fits were used to calculate the maximum flux (J_{max}) and the time of J_{max} (T_{max}) (El Maghraby et al., 1999).

The pseudo–steady state permeation profile was used to calculate the flux and the lag time by taking the slope of the linear portion of the profile as the flux and the intercept of this line with the time axis as the lag time (examples for individual formulations are shown in Figs. 2 and 3).

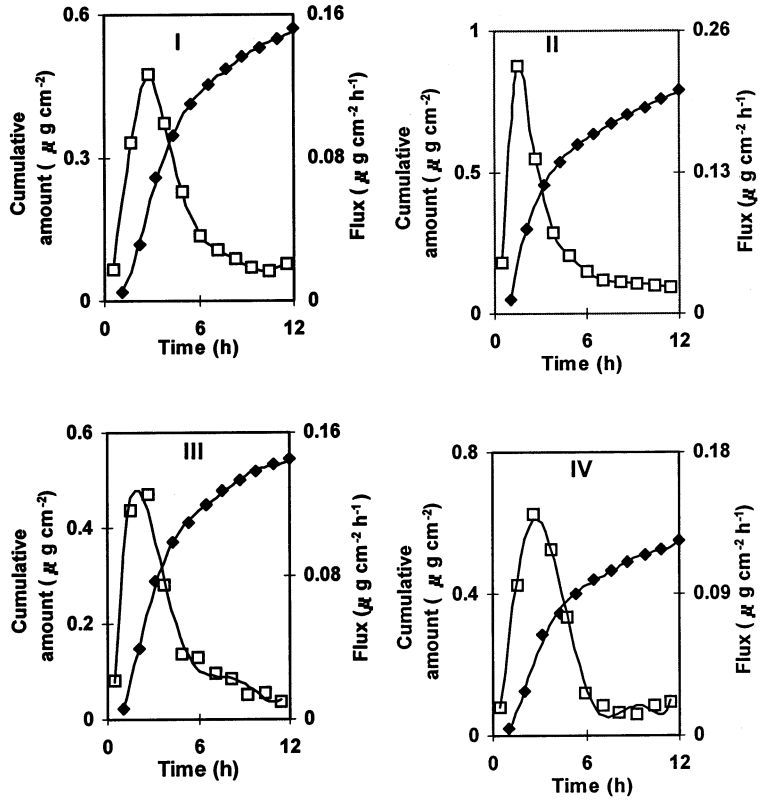

Fig. 1. Examples of the cumulative amount (◆) versus time plots and transepidermal flux profiles (□) of oestradiol from PC vesicles (I), sodium cholate (II) Span 80 (III) and oleic acid (IV) containing liposomes (low dose study).

When comparing lipid vesicles to lipid solutions, values relative to the corresponding control were used to overcome the difference in permeation profiles and to minimise the effects of skin variability.The Student t-test was used as the test for significance.

3. Results and discussion

3.1. Entrapment efficiency

The entrapment efficiency, expressed as percent of the starting drug, was determined after separation of the free drug by the mini-column centrifugation method. As previously shown (El Maghraby et al., 1999) the entrapment efficiency was high (93–99%), which is expected for a lipophilic compound like oestradiol. These results indicated that the amount of drug added was sufficient to saturate the lipids and thus maintain equal thermodynamic activities in all formulations. Thus, the maximum entrapment efficiency obtained was 99% for pure PC liposomes. Accordingly, at least 1% of the initial drug added remained free. This amount of free drug (at least 10 μg/ml) is above the saturation solubility of oestradiol in water.

3.2. Vesicle size

Photon correlation spectroscopy was used to measure vesicle size. As expected for vesicles prepared by sonication followed by manual extrusion, they were essentially homogeneous. They had a Z-Average mean diameter of 136 ± 3 nm ($n = 13$). There were no significant differences between different formulations as expected with such a method of preparation.

3.3. Skin delivery of oestradiol from lipid vesicles and lipids in solution

The delivery of oestradiol from traditional and deformable liposomes as well as the mechanisms of delivery were previously investigated (El Maghraby et al., 1999) and it was concluded that for maximum effect the drug should be applied concurrently with the liposomal ingredients. However, the work did not clarify whether or not liposomal structure was essential. From those previously tested formulations, three (I–III) were selected for the present study to test the importance of vesicular structure: pure PC vesicles (I) which produced a penetration enhancing effect; and sodium cholate and Span 80 which were separately incorporated into PC vesicles to form deformable vesicles (II, III) and which delivered the most drug. In addition, oleic acid, a well known penetration enhancer, was included instead of surfactants (Formula IV) in a trial to clarify the penetration enhancing effect (if any) of lipid vesicle components when compared with a known penetration enhancer.

To study the importance of intact lipid vesicles for skin delivery of oestradiol it was necessary to employ a solvent to solubilise the liposome components so as to compare vesicular delivery with delivery from solution containing the same components of the corresponding vesicles. Although ethanol was a possibility, it was avoided because of its drastic effect on the skin which could possibly mask the enhancing effect of any additive (Megrab et al., 1995a; Yokomizo, 1996a). Propylene glycol has been used as a solvent for phospholipids (Yokomizo, 1996a,b; Yokomizo and Sagitani, 1996a,b), it is a well-accepted adjuvent in topical formulations and its enhancing effect on skin permeation arising from structural changes is marginal (Yamane et al., 1995). It was therefore, selected as a vehicle to study the effects of vesicle components in solution. However, the solubility of oestradiol in pure propylene glycol is 75 mg/g (Salole, 1986) at 22°C and will be higher at 32°C,

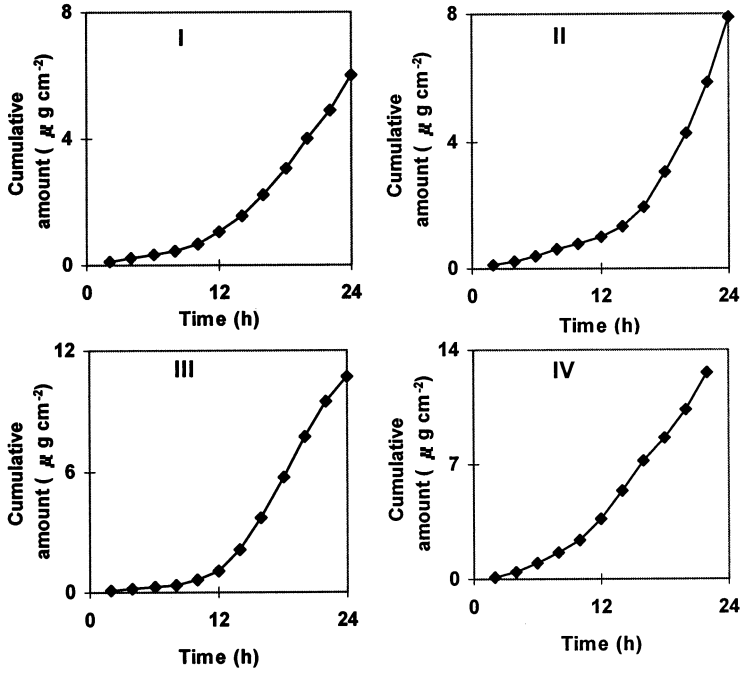

Fig. 2. Examples of pseudo–steady state transepidermal permeation profiles of oestradiol from saturated solutions in 90% w/w propylene glycol/water containing the components of PC liposomes (I), cholate containing vesicles (II), Span containing vesicles (III) and oleic acid containing liposomes (IV).

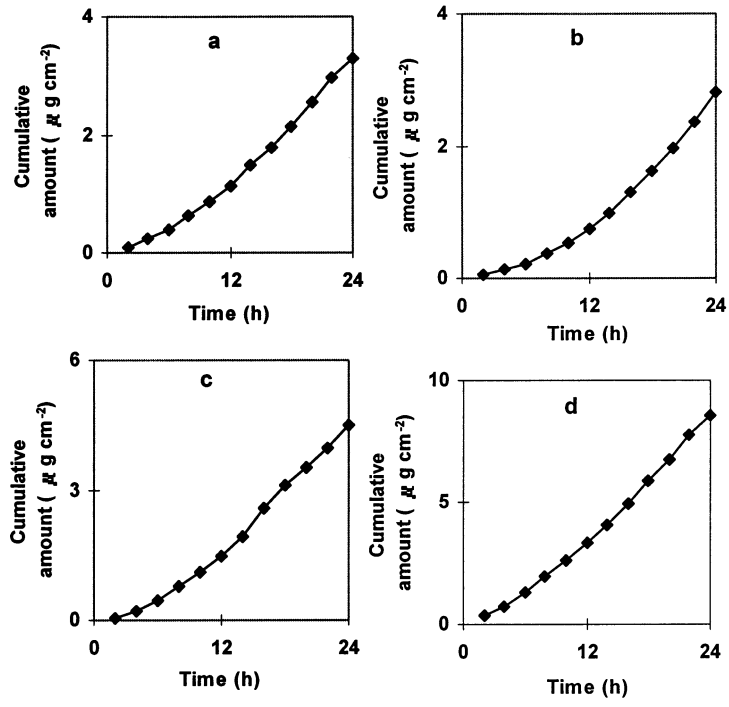

Fig. 3. Examples of pseudo–steady state transepidermal permeation profiles of oestradiol from saturated solutions in 90% w/w propylene glycol/water containing 0.7% w/v sodium cholate (a), 0.665% w/v Span 80 (b), 0.8% w/v oleic acid (c) or 7% v/v ethanol (d).

thus requiring much expensive radiolabel. Accordingly, 90% w/w propylene glycol in water was used (oestradiol solubility determined to be 9.6 mg/g at 32°C).

Vesicles were prepared as 5% w/v aqueous dispersions. The results of skin delivery of oestradiol from lipids in vesicles and solution form are presented in Table 2. Delivering oestradiol from saturated aqueous solution (CV) resulted in J_{max} values ranging from 8.34 to 11.5 ng/cm^2 per h (Table 2) in agreement with published data (Megrab et al., 1995a). Ideally, delivering drug from saturated solutions in different solvents (equal thermodynamic activity) should provide the same flux unless the solvent has an effect on the skin. As propylene glycol only marginally changes skin structure (Yamane et al., 1995), it might be expected that saturated oestradiol solution in 90% propylene glycol/water will give a flux close to that for oestradiol released from aqueous solution. However, the flux values obtained were in the range of 72.8–286 ng/cm^2 per h (Tables 2 and 3). These high values agree with those reported (Megrab et al., 1995b) and arise because propylene glycol enters the skin, increasing its partitioning properties for oestradiol and thus increasing the flux. This process also explains the longer lag times (4.7–6.6 h) as the skin dynamically readjusts to the presence of propylene glycol.

Pure PC vesicles (I) significantly improved ($P < 0.01$) the transepidermal flux of oestradiol but they did not affect the time of the maximum flux compared with the control, CV (Table 2). Applying the same formulation in the form of a lipid solution in which the drug was saturated also significantly increased ($P < 0.05$) oestradiol flux compared with its control, CS. This indicates the penetration enhancing effect of PC in solution, supporting our previous work (El Maghraby et al., 1999), and also correlates with reported data (Yokomizo, 1996a,b; Yokomizo and Sagitani, 1996a). The lag time was nearly doubled com-

Table 2
Skin delivery of oestradiol from lipid vesicles and and their components dissolved in 90% w/w propylene/water[a]

Vesicles				
Formula	Control (CV)		Test (medicated vesicles)	
	J_{max} (ng/cm^2 per h)	T_{max} (h)	J_{max} (ng/cm^2 per h)	T_{max} (h)
I	11.5 (1.3, 12)	2.42 (0.20, 12)	98.8 (18, 9)	2.41 (0.16, 12)
II	9.88 (1.1, 17)	2.25 (0.13, 17)	171 (17, 17)	1.88 (0.13, 17)
III	9.26 (1.6, 10)	2.22 (0.19, 10)	161 (33, 10)	1.96 (0.11, 10)
IV	8.34 (1.7, 4)	2.42 (0.10, 4)	108 (22, 5)	2.33 (0.27, 5)
Vesicle components in 90% w/w propylene glycol/water				
	Control (CS)		Test (lipid solution)	
	Flux (ng/cm^2 per h)	Lag time (h)	Flux (ng/cm^2 per h)	Lag time (h)
I	178 (87.6, 6)	6.63 (1.7, 6)	507 (104, 5)	12.4 (1.0, 5)
II	178 (87.6, 6)	6.63 (1.7, 6)	713 (261, 4)	12.0 (0.47, 4)
III	178 (87.6, 6)	6.63 (1.7, 6)	828 (199, 5)	12.1 (0.52, 5)
IV	72.8 (12.2, 10)	5.21 (0.78, 10)	500 (120, 9)	7.65 (0.86, 9)

[a] Values between brackets are SEM and *n*, respectively. CV is saturated aqueous solution; CS is saturated solution in 90% propylene glycol/water; I is PC liposomes; II is cholate, III is Span 80 and IV is oleic acid containing vesicles. Data from vesicle form (I–III) are quoted from El Maghraby et al. (1999).

pared with the control as further time was required for PC entry into the stratum corneum. We can compare the vesicle form with the solution form by ratioing flux values relative to the corresponding control (Fig. 4). Vesicles were found to be superior.

Cholate containing vesicles (II) increased the transepidermal flux of oestradiol by 17-fold (significantly different at $P < 0.001$) and reduced the time of maximum flux marginally by 17% compared with the saturated aqueous control. In solution form however, the vesicle components increased the flux 4-fold and increased the lag time by 82% compared with control (saturated solution in 90% propylene glycol/water). Again, vesicles were superior compared with lipid solution when comparing relative flux values (Fig. 4).

Span 80-containing vesicles (III) showed similar trends to cholate systems. They increased the transepidermal flux 17-fold (significantly different from control results at $P < 0.01$) but reduced the time of maximum flux marginally by 12% (considered to be a trend). When the vesicle components were used in solution form the flux was increased 4.7-fold (significantly different from control results at $P < 0.05$) and the lag time by 82%. Thus the vesicles are better than the solution form (Fig. 4).

Liposomes incorporating oleic acid increased the flux 13-fold (significantly different from con-

Table 3
Effect of individual liposomes components dissolved in 90% w/w propylene glycol/water on the skin delivery of oestradiol in vitro[a]

Variable	Test	Control
Oleic acid in solution		
Flux (ng/cm^2 per h)	520 (152, 7)	178 (87.6, 6)
Lag time (h)	7.83 (0.95, 7)	6.63 (1.7, 6)
Span in solution		
Flux (ng/cm^2 per h)	192 (26.1, 6)	178 (87.6, 6)
Lag time (h)	7.07 (1.5, 6)	6.63 (1.7, 6)
Cholate in solution		
Flux (ng/cm^2 per h)	155 (19.5, 3)	178 (87.6, 6)
Lag time (h)	7.04 (1.1, 3)	6.63 (1.7, 6)
Ethanol (7% v/v) in solution		
Flux (ng/cm^2 per h)	324 (84.1, 5)	286 (49.0, 5)
Lag time (h)	5.58 (0.27, 5)	4.73 (1.2, 5)

[a] Control — without oleic acid, Span or cholate.Values between brackets are SEM and *n*, respectively.

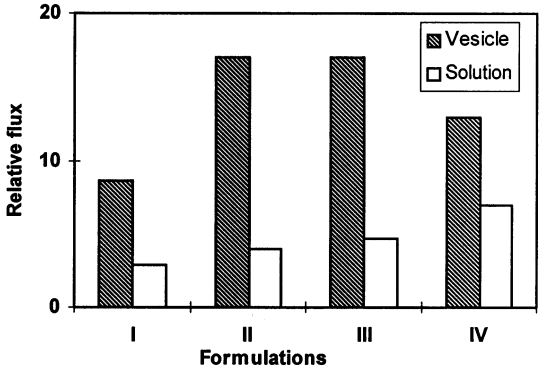

Fig. 4. Transepidermal fluxes relative to appropriate controls for oestradiol delivered from lipids in vesicle and solution forms. Formulation details of I–IV are in Table 1.

trol results at $P < 0.02$) with negligible effect on the time of maximum flux. Comparing these results with those obtained for surfactant-containing vesicles (II-III), it can be concluded that sodium cholate and Span 80 were even more efficient than the penetration enhancer oleic acid when incorporated in lipid vesicles. This further suggests that lipid vesicles increase oestradiol permeation by mechanisms that are additional to penetration enhancing effects. In solution form the components of liposomes incorporating oleic acid increased the flux by 6.9-fold (significantly different from control results at $P < 0.01$) and increased the lag time by 47% compared with the control. Again, the vesicular form was found superior to a lipid solution even after inclusion of a penetration enhancer such as oleic acid.

These results indicated that the transepidermal permeation of oestradiol is affected to a greater extent by the presence of lipids in aqueous environment compared to that in a 90% propylene glycol/water medium. However, lipids in aqueous medium were in the form of liposomes. These results thus further suggest that vesicular structure promotes skin delivery of oestradiol in vitro.

3.4. Influence of individual liposome components in skin delivery of oestradiol from solution form

The tested formulations included PC as the main lipid component. Sodium cholate, Span 80 and oleic acid were separately used as the second component in II-IV, respectively. In addition, 7% v/v ethanol was included in formulations II-IV. The effect of each of these second components in 90% propylene glycol on skin delivery of oestradiol was studied. The relative fluxes were considered together with that of pure PC and compared with that of the parent formula (Fig. 5).

Sodium cholate and Span at the tested concentrations had little effect on the skin delivery of oestradiol (Table 3). They resulted in relative flux values of 0.87 and 1.1, respectively. Ethanol at the tested concentration (7% v/v) showed no significant ($P > 0.05$) enhancing effect as it provided a relative flux of 1.1. The effect obtained with ethanol is expected as it only produced significant enhancement for oestradiol skin delivery at higher concentrations (40–60% w/w in water), when used on both sides of the membrane (Megrab et al., 1995a). The parent formulations of these components (in solution form) produced relative fluxes of 4.0 and 4.7 compared with control (Fig. 5). This effect, when compared with that of individual components (PC, surfactants and ethanol), suggests a possible synergistic effect for surfactants and ethanol with PC.

The effect of skin pretreatment with 5% w/w oleic acid in propylene glycol on the skin delivery of oestradiol was reported (Goodman and Barry, 1988). In this study oleic acid enhanced the skin delivery of oestradiol 11.9-fold when using 50% aqueous ethanol in the donor and receptor side. This was reduced to 3.5-fold on using water on both sides. Oleic acid also increased the skin delivery of a highly lipophilic molecule, tetrahydrocannabinol, when incorporated with the drug in propylene glycol–ethanol cosolvent (Touitou and Fabin, 1988). The greatest effect was obtained with 3–10% oleic acid, with 1% producing less enhancing effect. In the present study, oleic acid at the tested concentration (8 mg/ml) increased the transepidermal flux of oestradiol 2.9-fold. The parent formula containing oleic acid, PC and incorporating 7% ethanol (in the solution form) resulted in a relative flux of 6.9 compared with control (CS).

Considering the effect of pure PC (2.9-fold) and comparing the combined effect of individual com-

ponents with the parent formulations (Fig. 5), the results reflected an additive effect for oleic acid with PC.

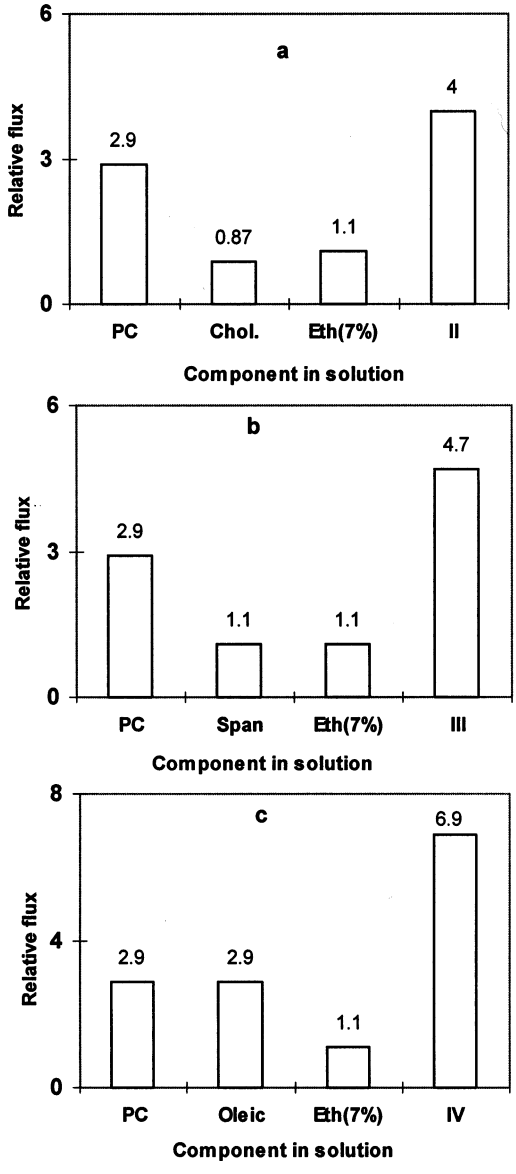

Fig. 5. Effect of individual components on relative flux of oestradiol from 90% propylene glycol/water solution (flux values were relative to saturated oestradiol in 90% propylene glycol/water). PC, phosphatidylcholine; Chol, cholate; Eth, ethanol and formulation details of II–IV are in Table 1.

4. Conclusions

Incorporation of surfactants sodium cholate or Span 80 into phosphatidylcholine liposomes was even better than incorporation of the penetration enhancer, oleic acid, for the skin delivery of the model lipophilic drug oestradiol. For optimum effects, the components should be in the form of vesicles. The overall results indicate that a penetration enhancing mechanism of liposome components is not the only or indeed the main factor operating. Liposome components in solution, however, have a definite additive effect with a possible synergism in some cases (surfactants or ethanol with PC).

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