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Influence of micelle solubilization by tocopheryl polyethylene glycol succinate (TPGS) on solubility enhancement and percutaneous penetration of estradiol

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Abstract

The effect of micellar solubilization on the enhancement of the solubility and percutaneous penetration of estradiol by the surface-active agent, tocopheryl polyethylene glycol succinate (TPGS) was characterized in this study. Results show that the solubility of estradiol was improved in the presence of TPGS through micellar solubilization. The critical micelle concentration (CMC) of TPGS increased with increasing ethanol concentration in the medium. With the flux corrected to the saturated level ($J_{corrected}$) of the free form of estradiol, an increase in the alcohol content of the medium resulted in an increase in $J_{corrected}$ for all levels of TPGS examined. For the same level of alcohol content, an increase in the TPGS concentration mostly led to a small extent of decrease in $J_{corrected}$. However, the extent of decrease was more obvious in media containing more than 60% alcohol. We also confirmed that only an insignificant amount of TPGS was transported across the skin (below the detection limit of 2 μg/ml). Permeabilities (P_{eff}), which describe the overall effects (DK/H) on the stratum corneum (SC), decreased with increasing TPGS concentration for media containing 0, 40, 60, and 80% alcohol, whereas they increased then decreased with increasing TPGS concentration for media containing 10 and 20% alcohol. The enhancement ratios based on P_{eff} assuming that the medium contained 0% TPGS and alcohol as unity did not increase accordingly with increases in TPGS concentration at the same level as alcohol. Likewise, the enhancement ratios for the same level of TPGS increased with low alcohol content, but then decreased with increasing alcohol content. We concluded that micellar solubilization by TPGS was able to improve the solubility of estradiol, but it only had an insignificant influence on the skin. Interfacial coverage of TPGS with increasing TPGS concentration and hindrance of the partitioning of estradiol by the increasing alcohol content might play a role in influencing the permeability of estradiol.
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Keywords: Estradiol; TPGS; Solubilization; Penetration; Micelles

1. Introduction

Topical delivery of drugs through the skin provides several advantages including avoidance of hepatic first-pass metabolism, reduction in side effects (such as gastric irritation by NSAIDs), better patient compliance, and enhanced therapeutic efficacy [1]. The suitability of many therapeutic agents for topical delivered is limited by the ability of the drugs

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to permeate the skin, in particular by the rate-limiting barrier of the stratum corneum [2]. Influencing factors can be theoretically summarized based on Fick's law ($J = DKCs/h$) that describes the flux (J) across a rate-limiting barrier (of thickness, h) at sink conditions including solubility (Cs), lipophilicity (partition coefficient, K), and the molecular weight or size (diffusion coefficient, D). Manipulation of these factors with structural modifications on parent drugs or with the aid of enhancers may improve topical delivery.

Manipulation of these influencing factors with the aid of enhancers has been the main focus in the past when developing topical dosage forms by improving the topical delivery of these drugs with acceptable physicochemical properties but without reaching excessive therapeutic levels. Enhancers play roles mostly of improving the thermodynamic activity for penetration by increasing drug solubility (Cs) [3,4], of promoting diffusion by altering the skin structure (D) [5–7], of modifying partition phenomena by transforming the barrier to be more lipophilic (K) [8,9], and of enhancing the flux by a simultaneous combination of several of the above mechanisms [10–12]. Since alterations and transformations of the skin structure can potentially lead to permanent injury to the important protection barrier of the skin, improvements in drug solubility with the aid of enhancers might be a better choice.

The two most commonly used enhancers for improving drug solubility are ethanol and propylene glycol. Both have dual functions as so-called cosolvents for increasing drug solubility and as chemical enhancers for improving skin permeability. Ethanol is currently contained in commercial transdermal delivery systems for estradiol [13] and fentanyl [14] as a cosolvent to increase drug solubility and as an enhancer by partitioning into and interacting with skin constituents to induce a temporary and reversible increase in skin permeability. Propylene glycol is widely used as a cosolvent to increase the solubility of lipophilic drugs and as a potential enhancer by increasing the solution capacity within the stratum corneum [15]. At least, both increase the thermodynamic activity of drugs by increasing drug solubility for penetration which enhances the flux.

In addition to solubility enhancement with a cosolvent to increase the thermodynamic activity of a drug for penetration, solubilization by various means such as eutectic formation with terpenoid derivatives [16], inclusion complex formation with cyclodextrin derivatives [17], entrapment in liposomes [18], and micelle formation with surface-active agents [19] has been recommended. Among these, only increased drug solubility by eutectic formation leads to all solubilized solutes being available for penetration, whereas the free form (not a complex or a portion encapsulated in liposomes or micelles) of solubilized solutes by the other three methods is deliverable to the skin. This was confirmed by the fact that complexation of prostaglandin E_1 (PGE_1) with β-cyclodextrin decreased the release rate of PGE_1 from a base containing HPE-101 [17]. This complexation did not increase the thermodynamic activity of the drug for penetration, but the resulting negative effect was well compensated for by an increase in partitioning of the released PGE_1 to the skin by HEP-101. A free drug mechanism whereby the drug is released from the liposome vehicles and then independently permeates the skin is considered one of five possible mechanisms by which different lipid vehicles can improve skin delivery of estradiol [18]. Investigations to delineate the role of surfactants in diffusional transport revealed that several factors including the thermodynamic activity of the solute, diffusivities of the free solutes and micelles, etc. must be considered. The importance of determining and defining the thermodynamic activity of the diffusing solute was emphasized [19].

Tocopheryl polyethylene glycol succinate (TPGS) is a water-soluble derivative of a natural source of vitamin E and functions as a surfactant with an HLB value of 13.2. Several studies have demonstrated that TPGS improves the oral bioavailability of vitamin E [20] and cyclosporin [21]. It was suspected that the enhancement of bioavailability is due to enhanced solubility, improved permeability, and reduced intestinal metabolism [22,23]. It was also reported that TPGS increases the oral absorption flux of amprenavir (an HIV protease inhibitor) by enhancing its solubility and permeability [24]. This was due to TPGS significantly improving the solubility of amprenavir through micelle solubilization. However, it was also revealed that the reduction in apparent permeability from the apical to the basolateral above the CMC of TPGS could be explained by the

reduced concentration of free amprenavir in the apical solution. Since it was evident that utilization of TPGS improved the solubility by micelle solubilization which enhanced the absorption flux, extension to potential improvements in topical delivery through the skin was explored. Estradiol was selected as the model drug since it has limited water solubility, and it is a drug for which improving the solubility for enhancing skin permeability is desired.

2. Experimental methods

2.1. Solubility measurements

An excess amount of estradiol was added to alcoholic aqueous solutions (0–80%, v/v) containing 0–20% (w/v) TPGS, which were agitated at 37 °C for more than 72 h. The mixtures were filtered with 0.2-μm Anopore centrifuge tube filters by centrifuging at 37 °C and 13,000 rpm for 15 min. The supernatants were sampled and diluted. The concentration of estradiol was determined by HPLC analysis.

When the TPGS concentration is larger than the critical micellar concentration (CMC), a free form of solute as well as a micelle-bound form of solute exists in the solution. The equilibrium constant (K_a) for the free form of the solute and micelle-bound solute can be deduced as follows:

$$S_{total} = S_{free} + S_{bound} \quad (1)$$

$$k_a = \frac{S_{bound}}{S_{free} \cdot (SAA)_m} \quad (2)$$

where S_{total} is the total concentration of the solute; S_{free} is the concentration of the free form of the solute; S_{bound} is the concentration of the solute bound to the micelles; and $(SAA)_m$ is the total concentration of TPGS in the micelles and is equal to the concentration of TPGS minus CMC [24]. The relationship between S_{total} and $TPGS_t$ is deduced as follows:

$$\begin{aligned} S_{total} &= S_{free}[1 + k_a(SAA)_m] \\ &= S_{free} + k_a \cdot S_{free}(TPGS_t\text{–}CMC) \\ &= S_{free}(1 - k_a CMC) + k_a \cdot S_{free} \cdot TPGS_t \end{aligned} \quad (3)$$

Based on the above equation, a linear plot of S_{total} versus $TPGS_t$ (total concentration of TPGS added to the solution) beyond the CMC was constructed. S_{free} was obtained by adding the intercept of the linear plot to the product of the slope and the CMC value (estimated from the plot). The equilibrium constant (K_a) was then calculated by dividing the slope by S_{free}.

2.2. Percutaneous penetration studies

An in vitro penetration study was conducted with Franz diffusion cells (membrane surface area of 2.54 cm^2 and a cell volume of 4.5 ml) using nude mouse skin as the main barrier. A 0.06% (w/v) estradiol-in-alcohol aqueous solution (0, 10, and 20%, v/v) containing 0–15% (w/v) TPGS was placed on the donor side, and phosphate-buffered saline solution (pH 7.4)–PEG 400 (50:50, v/v) was used as the receptor medium; this was maintained at 37 °C with a stirring rate of 500 rpm. At predetermined time intervals, 200-μl aliquots were withdrawn from the receptor compartment and replaced with an equal volume of fresh medium. The concentration of estradiol was determined by HPLC analysis.

The cumulative amount in the receptor was calculated by the following equation: where $M(t_n)$ is the penetrated amount per unit area; $C(t_n)$ is the concentration measured in the sample; V is the receptor volume; V_s is the sample volume; V_d is the volume of dilution to adjust drug concentration within the linear range of measurement; A is the surface area for penetration; and the subscript x refers to the summation index. The flux (J_m) is calculated from the linear portion of the plot of M_t versus time t.

$$M(t_n) = \left[C(t_n)V + (V_s + V_d)\left(\sum_{x=0}^{n-1} C(t_x) \right) \right] / A \quad (4)$$

According to Fick's law with the assumption that only free solute is available for partitioning into and diffusion through the skin barrier, the flux (J_m) across a barrier membrane at steady state calculated from the linear portion of $M(t)$ versus time plots is expressed as follows:

$$J_m = \frac{D}{H}[(S_{free})_{md} - (S_{free})_{mr}] \quad (5)$$

where D is designated as the diffusion coefficient of the solute in the barrier membrane; H is the thick-

ness of the barrier membrane; and $(S_{free})_{md}$ and $(S_{free})_{mr}$ are defined as the free solute concentration at the donor surface and at the receptor surface inside the barrier membrane, respectively. Since $(S_{free})_{md}$ is regarded as being greater than $(S_{free})_{mr}$, sink conditions are applicable in this study. Simplification produces the following equation:

$$J_m = \frac{D}{H}(S_{free})_{md} \quad (6)$$

The partition coefficient (K) between the solution phase and the barrier membrane phase is defined as follows:

$$K = \frac{(S_{free})_{md}}{S_{free}} \quad (7)$$

Substitution of K with the definition of P_{eff} being equal to KD/H yields:

$$J_m = \frac{KD}{H}(S_{free}) \quad (8)$$

$$P_{eff} = \frac{KD}{H} \quad (9)$$

S_{free} is designated as the free solute concentration in the donor compartment. P_{eff} is referred to as the effective permeability of the free solute across the skin membrane, and P_{app} is defined as the permeability with respect to the total concentration of the solute in the donor compartment.

$$J_m = P_{app} \cdot S_{total} \quad (10)$$

$$\therefore P_{app} = \frac{J_m}{S_{total}} \quad (11)$$

According to Eq. (11), P_{app} is calculated by knowing J_m and S_{total} (total solute concentration in the donor compartment). As defined by Eq. (12), P_{eff} is then calculated by multiplying P_{app} with the equilibrium constant (K_a) and the micellar concentration of TPGS (as $(SAA)_m$) obtained in the solubility measurement section.

$$S_{total} = S_{free} + S_{bound}$$

$$S_{total} = S_{free} \cdot [1 + k_a(SAA)_m]$$

$$P_{app} = \frac{J_m}{S_{total}} = \frac{S_{free}}{S_{total}} \cdot \frac{KD}{H}$$

$$= \frac{KD}{H} \cdot \frac{1}{[1 + k_a(SAA)_m]} = \frac{P_{eff}}{[1 + k_a(SAA)_m]}$$

$$P_{eff} = P_{app}[1 + k_a(SAA)_m] \quad (12)$$

However, the solute concentration was kept the same for all media compared. Because of this, the amount of free drug available for penetration differed. The corrected flux ($J_{corrected}$) is defined as the flux with respect to the solute concentration at a saturated state and is expressed by Eq. (13). S_{sat} is designated as the saturated concentration of the free form of solute in the corresponding medium.

$$J_{corrected} = \frac{J_m \cdot S_{sat}}{S_{free}} \quad (13)$$

2.3. HPLC analysis of estradiol

Estradiol was determined using an HPLC method with a reverse-phase ODS-2 column. Measurements were taken with fluorescence detection (excitation at 280 nm and emission at 312 nm). The mobile phase consisted of acetonitrile–H_2O (60:40, v/v) and a delivery rate of 1 ml/min with the column oven set at 35 °C. This method was validated in the linear concentration range of from 0.05 to 2 μg/ml. Precision and accuracy for intraday and interday measurements were within acceptable ranges of 2.0–4.8 and 0.2–2.4%, respectively.

2.4. HPLC analysis of TPGS

TPGS was also determined by an HPLC method after saponification using a reversed-phase C8 column (Lichrospher 250-4, 5 μm, Merck, Germany). Measurements were taken with UV detection at a wavelength of 284 nm. The mobile phase consisted of methanol–10 mM phosphoric acid (95:5, v/v) at a delivery rate of 1 ml/min. Vitamin E acetate was used as the internal standard. This method was validated in the linear concentration range of from 2 to 100 μg/ml. Precision and accuracy for intraday and interday measurements were within acceptable ranges.

Measurement of TPGS concentrations followed the method reported by Traber et al. [25], in which 2.5 ml of standard solution (2, 5, 10, 25, 50, and 100 μg/ml TPGS in a 0.2% phthalein alcoholic solution)

or sample solution (from the receptor compartment after 96 h of the penetration study with 0.8 ml of a 15% TPGS solution in the donor compartment) was supplemented with 50 mg of ascorbic acid, 25 ml of a phthalein alcoholic solution, and two to three pieces of boiling stones in a round-bottomed flask. The mixture was continuously refluxed at a temperature of 100–150 °C in a oil bath until completely dissolved. Then, 0.25 g of potassium hydroxide was added, and reflux was continued for at least 30 min. After removing the flask from the oil bath, 1–2 ml of HCl, diluted with 25 ml deionized water, were added along the flask wall, and we ensured that the mixture solution did not appear pink (if the solution was pink, more HCl was added to neutralize the solution). The resulting solution was mixed with 5 ml of the internal standard solution (25 μg/ml vitamin E acetate in iso-octane). Then 2 ml of the upper layer were sampled and blown until dry under N_2 gas. The residue was reconstituted with 2 ml of the mobile phase. Analysis of TPGS as vitamin E followed the HPLC conditions described above.

3. Results and discussion

It has long been known that an enhancer to improve permeation across the skin is mainly due to the increase of drug solubility as free form available for penetration as well as its partition into the skin to modify barrier function. Expectedly for such an enhancer as ethanol for several drugs in skin permeation, the flux increases with increasing fraction of ethanol in the solvent mixture as long as thermodynamic activity of drug increases with increasing ethanol content. However, a conflict in influence of ethanol partitioning into the skin to the enhancement of drug solubility with increasing alcohol content on the permeation should be the result. Therefore, a combination of ethanol and water as solvent would be preferably selected for testing the enhancement ability of TPGS on the skin permeation of estradiol with ethanol content as optimally as possible.

As known, TPGS is able to improve oral bioavailability either by increasing drug solubility as a result of micellar solubilization or by incorporating into cell membranes to disturb their integrity, resulting in the enhancement of drug absorption. Although it was expected that the improvement of drug solubility by micellar solubilization could mean that the thermodynamic activity of drug at a fixed concentration decreases with increasing TPGS concentration, TPGS as surfactant could effectively improve or enhance drug flux by the ability of decreasing the interfacial tension to make favorable partition of drug into the skin and of modifying the interfacial barrier function of stratum corneum to decrease the resistance for drug permeation.

The enhancement ability of ethanol via its influence on the skin barrier is also determined by the extent of ethanol penetrated into the skin. There expectedly exists an interaction between TPGS and ethanol, via which TPGS can modify the partition of ethanol between skin phase and solvent phase, leading to an interacting influence on the enhancement ability of ethanol by TPGS. Therefore, the influence on the drug permeation by adding various concentrations of TPGS in different ratios of ethanol in such a solvent of EtOH–water was examined and mutual effects were compared. Optimally, the interacting influence of ethanol and TPGS on the permeation mechanism of estradiol through the skin could be revealed.

Firstly, improvement in the solubility of estradiol by TPGS at different levels of alcohol content is examined. Results indicate that the solubility of estradiol was proportionally enhanced with an increasing concentration of TPGS beyond the critical micellar concentration (CMC). This was true for all levels of alcohol content. Furthermore, the addition of alcohol significantly enhanced the extent of improvement in estradiol solubility by TPGS, as indicated by the increase in the slope of the linear plots shown in Fig. 1. The slope was 0.0057 for medium containing 0% alcohol (Fig. 1A) and 0.1380 for that containing 80% alcohol (Fig. 1F). However, greater compliance to this linear relationship was observed for those systems containing a lower percentage of alcohol (0, 10, and 20%). The addition of alcohol seemed to obviously alter the CMC of TPGS in the alcohol–aqueous solution. This was estimated from the dramatic change in the solubility of estradiol with respect to the TPGS concentration, and results are listed in Table 2. The CMC for medium containing 0% alcohol was estimated to be

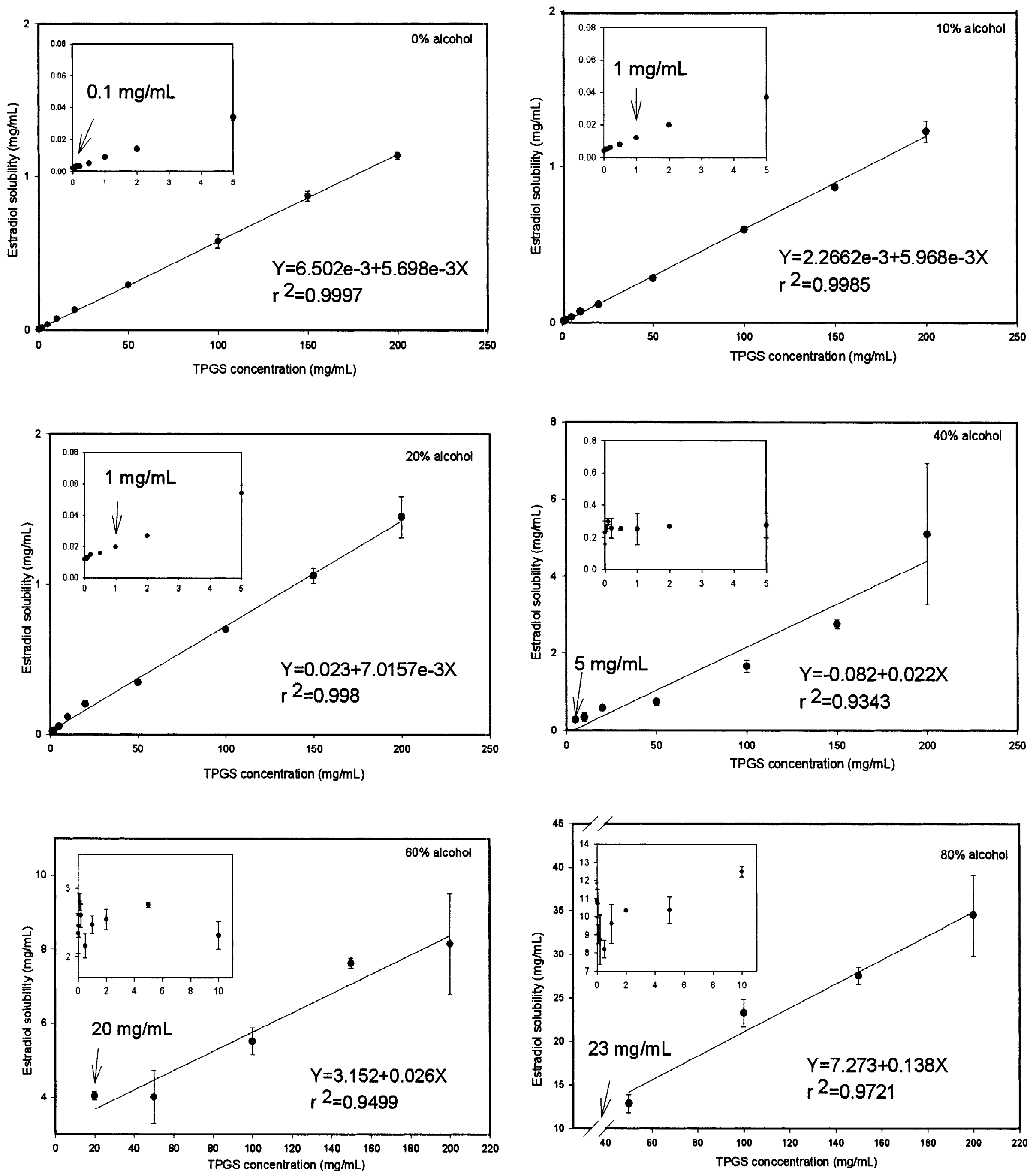

Fig. 1. The solubility profiles of estradiol in EtOH/TPGS cosolvent system. The data point and error bars represent the mean±S.D. of three replicates.

0.1–0.2 mg/ml, and that gradually increased to 23 mg/ml for medium containing 80% alcohol.

From the solubility measurements, the CMC of TPGS in deionized water at 37 °C was determined to be approximately 0.1–0.2 mg/ml which is a little lower than the literature values of 0.2 mg/ml [20,24] and 0.2–0.4 mg/ml [26] from surface tension measurements. Probably, this discrepancy might be attributed to the latter results being measured in phosphate buffer (pH 7) with an ionic strength of 0.15 M, while it was measured in pure deionized water in this study. Since the CMC can indicate the monomer concentration of a surface-active agent in the medium examined, the increase in the CMC of TPGS with increasing alcohol content in the medium may have been due to the increased monomer solubility of TPGS with increasing alcohol content. This was also reflected by the increase in the free form of the solute with increasing alcohol content.

With micelle solubilization above the CMC, the improvement in estradiol solubility with increasing TPGS was a result of an increase in micelle-bound solutes. By plotting total solubility of estradiol versus total concentration of TPGS added, the total concentration of free estradiol in the medium (S_{free}) and the equilibrium distribution coefficient (K_a) were calculated from the intercept ($S_{free}-S^{*}_{free}K^{*}_{a}(CMC)$) and the slope ($S^{*}_{free}K_a$) with the estimated CMC of the corresponding medium. These results are also listed in Table 1. They indicate that S_{free} slowly increased with increasing alcohol content in the medium of from 0 to 40% and then sharply increased with a further increase in the alcohol content to an extent higher than 60%. Correspondingly, the equilibrium distribution coefficient also decreased with increasing alcohol content in the medium. Fig. 2 illustrates this relationship between S_{free} or K_a and alcohol content. The increase in alcohol content in the solvent not only improved the solubility of the free form of estradiol but also modified the lipophilicity of the medium leading to a change in the equilibrium distribution of estradiol between the solvent phase and micelle phase of TPGS. Assuming a fixed lipophilicity for micelles of TPGS, the equilibrium distribution constant of estradiol expectedly decreased with increasing lipophilicity of the medium due to the increasing alcohol content. The micellar nature of TPGS, including the size of the micelles and number of TPGS monomers in each micelle, might be altered with increasing alcohol content, resulting in different extents of interfacial

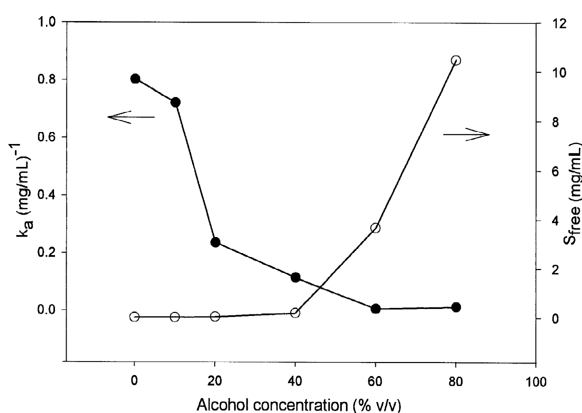

Fig. 2. The influence of different alcohol concentration on the free form concentration of solubilized estradiol (S_{free}) and equilibrium distribution coefficient in EtOH/TPGS cosolvent systems.

Table 1
CMC of TPGS, S_{bound}, S_{free} and K_a of estradiol in different EtOH/TPGS cosolvent system

Alcohol conc. (%, v/v)	TPGS CMC (mg/ml)	S_{bound} (mg/ml) TPGS concentration (w/v%)							S_{free} (mg/ml)	K_a (mg/ml)$^{-1}$
		0	0.01	0.1	0	5	10	15		
0	0.1	0	–	0.0019	0.0649	0.2879	0.5689	0.8649	0.0071	0.8028
10	1	0	0	0.0037	0.0647	0.2787	0.5857	0.8577	0.0083	0.7229
20	1	0	0	–	0.0864	0.3174	0.6714	1.0294	0.0296	0.2365
40	5	0	0	0	0.1375	0.5455	1.4675	2.5675	0.1945	0.1157
60	20	0	0	0	0	0.3281	1.8381	3.9561	3.6739	0.0071
80	23	0	0	0	0	2.3873	12.7703	17.0483	10.4827	0.0132

CMC, critic micelle concentration; S_{bound}, micelle-bound estradiol concentration; S_{free}, free estradiol concentration; and K_a, equilibrium distribution coefficient.

interactions that could also cause a decrease in the equilibrium distribution constant.

The cumulative amount of estradiol penetrating through mouse skin per unit area is illustrated in Fig. 3. The corresponding fluxes calculated from the linear portion of the plot are listed in Tables 2 and 3, which demonstrates that with a fixed concentration of estradiol (0.6%, w/v) in the medium, the flux was not accordingly enhanced with increasing TPGS concentration at the same level of alcohol content but had a tendency to be maximized at a concentration of TPGS dependent on the alcohol content in the medium. Furthermore, plots of flux versus alcohol at different TPGS concentrations illustrated in Fig. 4 demonstrate that the maximal enhancement of estradiol penetration by TPGS occurred at different concentrations for media containing different alcohol contents.

Since the free estradiol concentration varied at different compositions of ethanol and TPGS for a fixed estradiol concentration (0.6%), it would be more proper to compare the enhancement of the flux of ethanol and TPGS at its maximal thermodynamic activity. The corrected flux ($J_{corrected}$) was calculated according to the correction of the flux at an unsaturated free drug concentration relative to that at its saturated free drug concentration. The results are plotted in Fig. 5, which clearly indicates that the increase in alcohol content in the medium resulted in an increase in the corrected flux for all levels of TPGS examined. For the same level of alcohol content, the increased TPGS concentration mostly led to a small decrease in the corrected flux. However, the extent of decrease became more obvious for media containing greater than 60% alcohol. A study by Bommannan et al. [27] described how delipidation of the stratum corneum by a low alcohol content resulted in enhancement of the flux, whereas it was due to dehydration by alcohol at a high alcohol content in a report by Megrab et al. [28]. Perhaps TPGS produces a different interaction which interferes with either the delipidation or dehydration by alcohol resulting in these phenomena.

P_{eff} is defined as the overall effects (DK/H) of components in the medium on the permeability of free solute through the main barrier of the stratum corneum. Assuming P_{eff} for medium containing 0% TPGS and 0% alcohol to be unity, the enhancement ratios were calculated for the remaining media, and results are listed in Table 4. Results show that enhancement ratios for alcohol contents of 0% and higher than 20% were lower than 1, and then values decreased with increasing TPGS amount. In contrast for alcohol contents of 10 and 20%, both enhancement ratios were maximized with 0.01% TPGS at values larger than 1, and values then decreased with increasing TPGS amount. This also indicates that the mutual effect of alcohol and TPGS on the enhancement was maximized for the 20% alcoholic aqueous solution with the addition of 0.01% of TPGS. This is reflected in Fig. 6.

The overall effects of TPGS and alcohol on the SC were further elucidated by measuring the appearance of TPGS through the SC in the receptor compartment. Fig. 7A shows the chromatographs which detected vitamin E (peaks at 8 min) after saponification of TPGS in standard solutions containing a series of TPGS concentrations. Vitamin E acetate was used as the internal standard (peaks at 9 min). Fig. 7B,C demonstrates the results of assaying either a sample from the receptor compartment after the 96-h penetration study or the same sample with saponification, respectively. This clearly indicates that TPGS did not penetrate the SC in its intact form or as its degradation product of vitamin E. Knowing this and with judgement based on its molecular weight and high HLB value of 13.2, partitioning of TPGS into and its retention in the SC are expected to be minimal. The influence of TPGS on the permeability of estradiol through the SC might be attributed to its modification of estradiol's solubility and hence the interfacial interaction and partition phenomena. Expectedly, this should greatly differ from the effect of alcohol, which is permeable across the SC.

Since the SC is permeable to alcohol but not to the monomer and micellar forms of TPGS, the partitioning process for alcohol should be included for consideration. Increasing the TPGS concentration would predominately increase the number of micelles and the interfacial excess of TPGS between the SC phase and the medium, but not the characteristics of the micelle phase for the equilibrium distribution of estradiol and that of the medium for partitioning into the SC. Two possible influences on these partition processes might result at the same

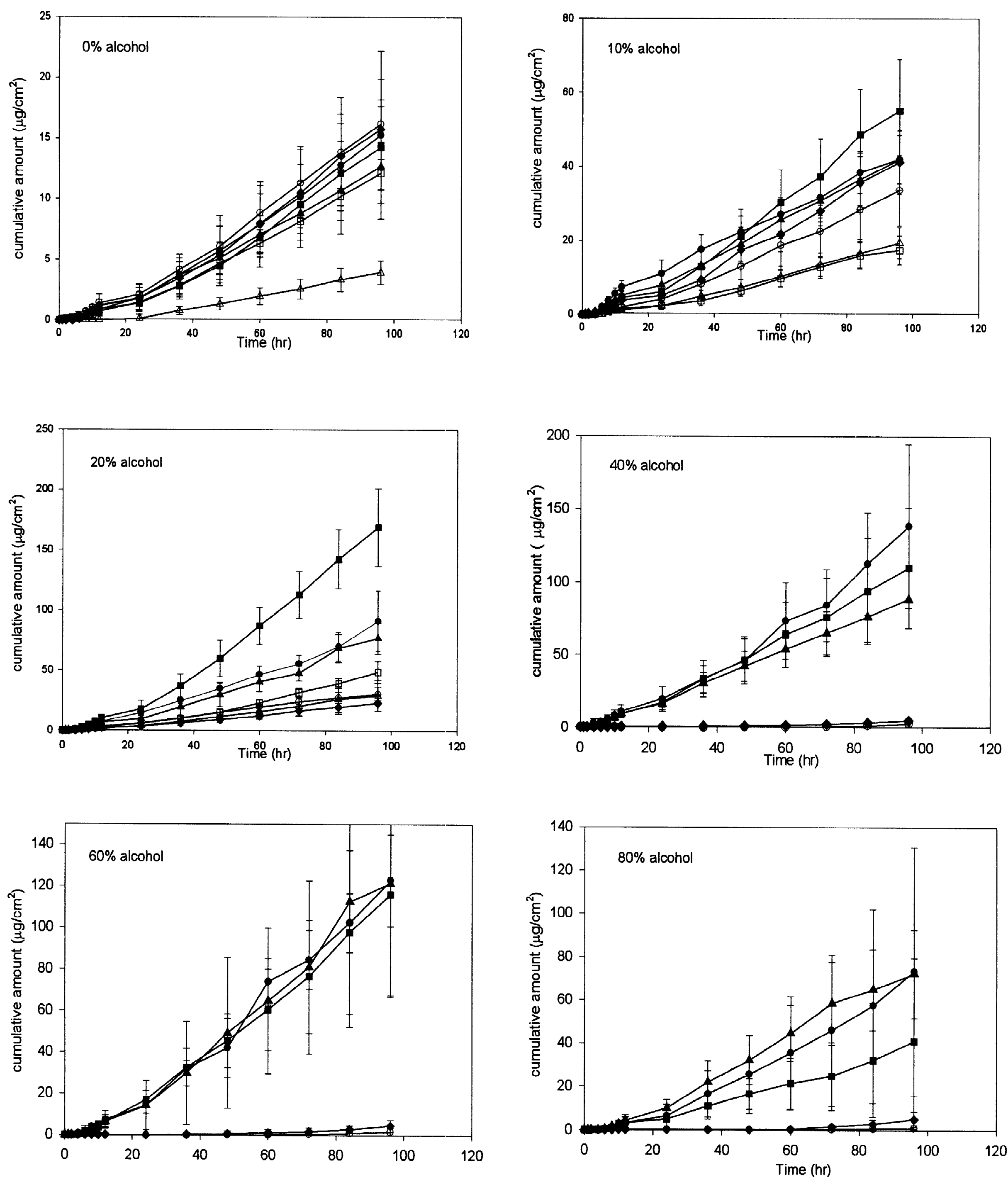

Fig. 3. The effect of EtOH/TPGS cosolvent systems on the in vitro transport of estradiol through nude mouse skin. Each data point is the mean±S.D. of five determinations. Key to TPGS concentration: (●) 0%; (■) 0.01%; (▲) 0.1%; (♦) 1%; (○) 5%; (□) 10%; (△) 15%.

Table 2

J_m (μg/cm^2 per h), S_{total} (mg/ml), S_{free} (mg/ml), P_{app}(cm/h), and P_{eff} (cm/h) of estradiol in EtOH/TPGS cosolvent systems containing 0, 10 and 20% alcohol

TPGS (%, w/v)	0% Alcohol					10% Alcohol					20% Alcohol				
	J_m	S_{total}	S_{free}	P_{app}	P_{eff}	J_m	S_{total}	S_{free}	P_{app}	P_{eff}	J_m	S_{total}	S_{free}	P_{app}	P_{eff}
0	0.211±0.002	0.002	0.002	0.1055	0.1055	0.471±0.057	0.004	0.004	0.1178	0.1180	1.48±0.166	0.012	0.012	0.1237	0.1240
0.01	0.207±0.004	0.003	0.007	0.0690	0.0690	0.698±0.018	0.005	0.005	0.1369	0.1400	2.279±0.025	0.013	0.0	0.1753	0.1750
0.1	0.161±0.002	0.009	0.007	0.0179	0.0308	0.453±0.008	0.012	0.008	0.0378	0.0623	0.951±0.051	0.020	0.013	0.0476	0.0480
1	0.219±0.005	0.072	0.007	0.0030	0.0272	0.547±0.024	0.073	0.008	0.0075	0.0611	0.283±0.010	0.116	0.030	0.0024	0.0076
5	0.205±0.003	0.295	0.007	0.0007	0.0285	0.463±0.017	0.287	0.008	0.0016	0.0598	0.338±0.013	0.347	0.030	0.0010	0.0123
10	0.162±0.003	0.576	0.007	0.0003	0.0228	0.240±0.011	0.594	0.008	0.0004	0.0296	0.696±0.012	0.600	0.025	0.0012	0.0283
15	0.055±0.001	0.600	0.005	0.0001	0.0111	0.243±0.002	0.600	0.006	0.0004	0.0443	0.362±0.010	0.600	0.017	0.0006	0.0219

Table 3

J_m (μg/cm^2 per h), S_{total} (mg/ml), S_{free} (mg/ml), S_{free} (mg/ml), P_{app} (cm/h), and P_{eff} (cm/h) of estradiol in EtOH/TPGS cosolvent systems containing 40, 60 and 80% alcohol

TPGS (%, w/v)	40% Alcohol					60% Alcohol					80% Alcohol				
	J_m	S_{total}	S_{free}	P_{app}	P_{eff}	J_m	S_{total}	S_{free}	P_{app}	P_{eff}	J_m	S_{total}	S_{free}	P_{app}	P_{eff}
0	2.26±0.062	0.231	0.231	0.0098	0.0098	1.587±0.061	0.600	0.600	0.0026	0.0026	0.899±0.036	0.600	0.600	0.00150	0.00150
0.01	1.408±0.051	0.297	0.297	0.0047	0.0047	1.570±0.060	0.600	0.600	0.0026	0.0026	0.669±0.027	0.600	0.600	0.00112	0.00112
0.1	0.963±0.006	0.253	0.253	0.0038	0.0038	1.544±0.072	0.600	0.600	0.0026	0.0026	0.573±0.020	0.600	0.600	0.00096	0.00096
1	0.089±0.006	0.332	0.195	0.0003	0.0004	0.118±0.014	0.600	0.600	0.0002	0.002	0.124±0.015	0.600	0.600	0.00021	0.00021
5	0.092±0.012	0.600	0.097	0.0002	0.0010	0.048±0.009	0.600	0.495	0.001	0.0001	0.019±0.001	0.600	0.442	0.00003	0.00004

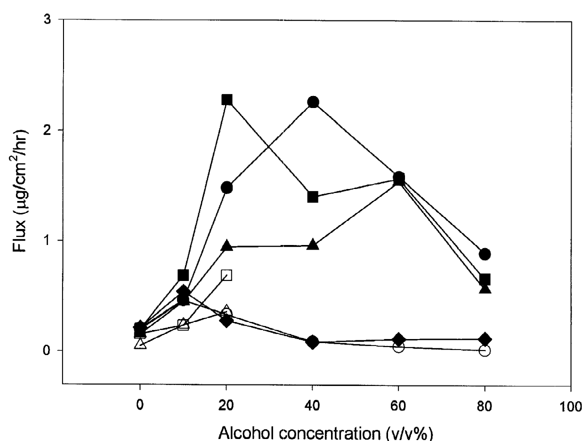

Fig. 4. The influence of EtOH/TPGS cosolvent systems on the flux (J) of estradiol through nude mouse skin (key is referred to in Fig. 3).

alcohol content. One is that the thermodynamic activity of alcohol decreases with increasing TPGS concentration. This possibly occurs through micellar solubilization or alteration of the lipophilicity of the medium, which leads to a lesser extent of modification of the lipophilicity of the SC for estradiol partitioning and of the diffusion pathway for estradiol diffusion by the penetrating alcohol. The other is due to the surface-active property of TPGS in which the barrier hindrance between the SC phase and the medium increases the partitioning with increasing TPGS concentrations as a result of interfacial coverage of the intervening TPGS molecules.

Table 4
Enhancement ratio of estradiol in different EtOH/TPGS cosolvent system

TPGS (%, w/v)	Enhancement ratio					
	0	10	20	40	60	80
0	1	1.113	1.170	0.092	0.025	0.0142
0.01	0.651	1.321	1.651	0.044	0.025	0.0106
0.1	0.291	0.588	0.453	0.035	0.025	0.0091
1	0.257	0.577	0.072	0.004	0.002	0.0020
5	0.269	0.564	0.116	0.009	0.001	0.0004
10	0.215	0.279	0.267	–	–	–
15	0.105	0.418	0.206	–	–	–

Along with these two mechanisms, the enhancement ratio at 0% alcohol content decreased with increasing TPGS concentration as a result of increasing interfacial coverage of TPGS for hindering the partitioning of estradiol into the SC. The enhancement ratio at higher alcohol contents (40, 60, and 80%) decreased with increasing TPGS even to an extent larger than that for 0% alcohol content, since both mechanisms can produce such a result. At 10 and 20% alcohol contents, the partitioning of alcohol was maximized at an appropriate concentration of TPGS to increase the enhancement ratio with increasing TPGS concentration; it then decreased as a result of increasing interfacial coverage of TPGS, which hindered the partitioning of estradiol into the SC.

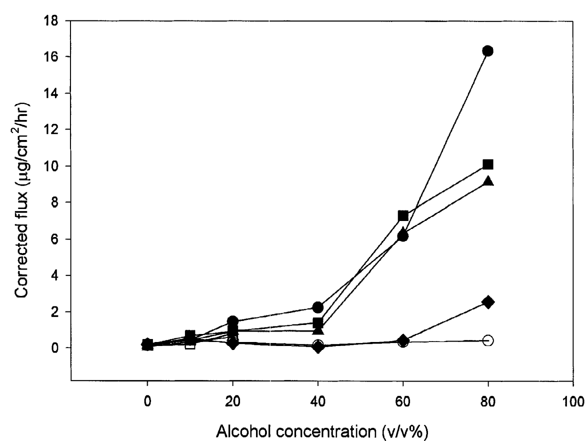

Fig. 5. The influence of EtOH/TPGS cosolvent systems on the corrected flux ($J_{corrected}$) of estradiol through nude mouse skin (key is referred to in Fig. 3).

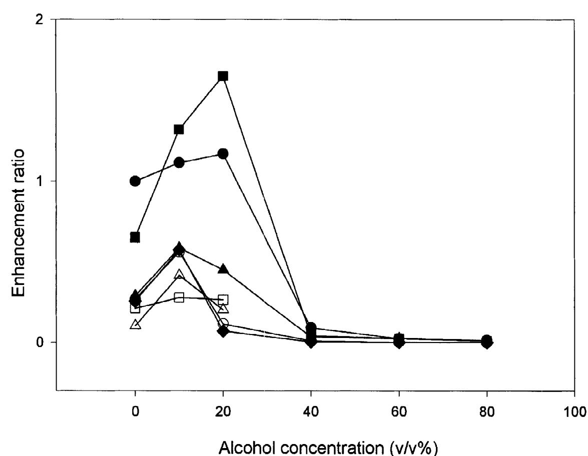

Fig. 6. Enhancement ratio of estradiol in different EtOH/TPGS cosolvent systems (key is referred to in Fig. 3).

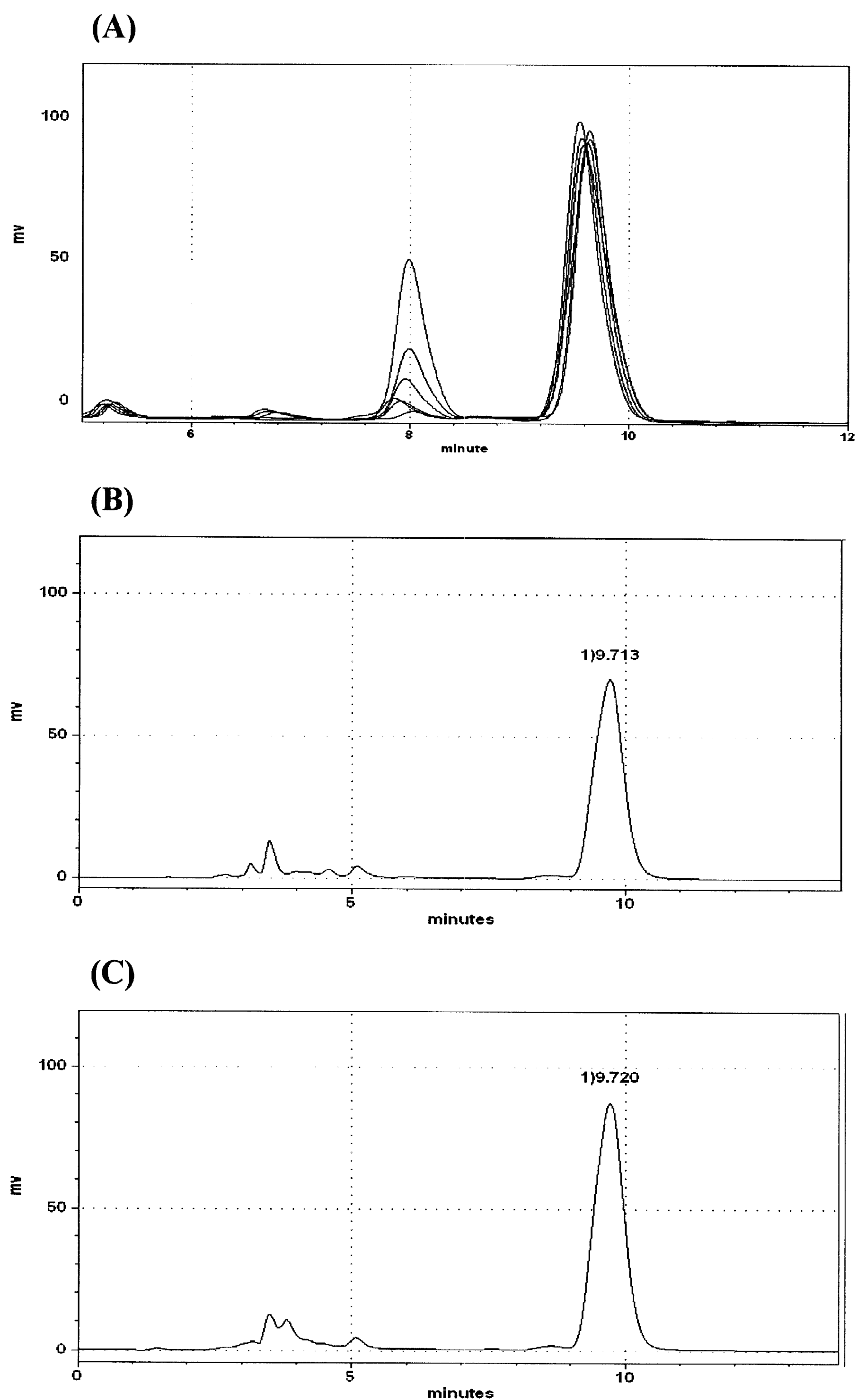

Fig. 7. Chromatographs of TPGS for standard samples (2–100 μg/ml) after saponification (A) and TPGS content in the receptor cell after penetration from the cosolvent system containing 15% TPGS determined by (B) direct measurement of vitamin E; (C) measurement of vitamin E after saponification.

4. Conclusions

TPGS was able to enhance the solubility of estradiol by micellar solubilization. But it was not responsible for the enhancement of estradiol penetration. On the other hand, alcohol was more effective in enhancing estradiol solubility by increasing the free-form concentration of estradiol and decreasing the equilibrium distribution constant. Modification of the skin to enhance estradiol penetration by TPGS was minimal compared to that by alcohol. The possible role of interfacial coverage by TPGS in hindering the partitioning of solute into the SC is worthy of detailed exploration in the future.

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