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Mechanistic studies of the effect of hydroxypropyl-β-cyclodextrin on in vitro transdermal permeation of corticosterone through hairless mouse skin

D.S. Shaker[a,*], A.-H. Ghanem[a], S.K. Li[a], K.S. Warner[a], F.M. Hashem[b], W.I. Higuchi[a]

[a] *Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, 30 South 2000 East, 213 Skaggs Hall, Salt Lake City, UT 84112, USA*
[b] *Department of Pharmaceutics, Helwan University, Cairo, Egypt*

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Abstract

Literature reports reveal that the issue of whether cyclodextrins may act as skin permeation enhancers has not been resolved. Accordingly, in vitro skin transport studies were conducted to address this question. Corticosterone (^{3}H-CS and/or non-radiolabeled CS) was chosen as the model permeant for transport experiments with hairless mouse skin (HMS) and with a synthetic cellulose membrane of 500 molecular weight cut off (MWCO), the latter to help establish baseline behavior. Hydroxypropyl-β-cyclodextrin (HPβCD) was selected as the representative cyclodextrin. The CS/HPβCD complexation constant was determined both from solubility data (saturation conditions) in phosphate buffered saline (PBS), pH 7.4 and with data obtained from PBS/silicone polymer partitioning experiments, the latter experiments permitting the determination of the complexation constant at low CS concentrations. These results were used in the calculations of the free CS concentrations in the donor chamber of the transport experiments. The CS transport experiments were conducted at CS solubility saturation and under supersaturation (resulting from autoclaving at 121 °C) conditions as well at very low (tracer level) concentrations. The effect of polyvinylpyrrolidone as a solution additive was also evaluated. The following were the key outcomes of this study. Contrary to literature reports, there was no evidence that HPβCD is an enhancer for CS transport through HMS. The CS permeability coefficient values obtained with HMS in all of the experiments were found to be the same within experimental error when calculated on the basis of the free CS concentration as the driving force for permeation. The constancy of the permeability coefficient in the presence and absence of HPβCD is interpreted to mean that, in these experiments, HPβCD did not alter the barrier properties of HMS stratum corneum to any significant extent nor did it enhance CS transport in any other manner such as by a carrier mechanism involving the aqueous boundary layer or by a carrier mechanism within the stratum corneum.

Keywords: Hydroxypropyl-β-cyclodextrin; Permeability coefficient; Supersaturation; Hairless mouse; Skin; Enhancer

1. Introduction

For the past several years, our laboratory has been involved in studying the mechanisms of action and the structure–function relationships of chemical skin permeation enhancers. Recent efforts have been focused

* Corresponding author. Tel.: +1-801-585-1268; fax: +1-801-585-1270.
E-mail address: dalsamuel@hotmail.com (D.S. Shaker).

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on studying several homologous series of *n*-alkyl permeation enhancers with the aim of factoring out the contributions of the polar head group and the *n*-alkyl group of the enhancer to the enhancer potency (Kim et al., 1992a; Yoneto et al., 1995a; Warner et al., 2001). An important part of the research plan has been to also consider highly lipophilic enhancers in our studies, including enhancers with relatively long *n*-alkyl groups (≥carbon number of 12). As such enhancers would generally possess rather low water solubilities, it is necessary in this situation to use solubilizing agents for the enhancers in order to carry out meaningful quantitative skin permeation studies. A desired feature of any solubilizing agent would be that the agent itself should not be a skin permeation enhancer. Should the solubilizing agent contribute importantly to the skin permeation enhancement of a drug molecule, quantitation of the potency of the studied enhancer can prove to be difficult.

For the purpose of solubilizing lipophilic skin permeation enhancers in aqueous media in our permeation enhancement studies, we have elected to consider a cyclodextrin derivative, hydroxypropyl-β-cyclodextrin (HPβCD). The cyclodextrins are a well investigated class of pharmaceutical excipients that are quite effective for solubilizing lipophilic compounds in aqueous media (Duchene and Wouessidjewe, 1993; Loftsson and Brewster, 1996; Rajewski and Stella, 1996; Stella and Rajewski, 1997). While we had thought at the beginning of our studies that cyclodextrins may be well suited for this intended purpose, a review of the literature revealed that the issue of whether cyclodextrins might themselves be skin permeation enhancers had not been resolved. Some investigators have reported that HPβCD may extract lipids from the stratum corneum and thus may increase the permeation of drugs through skin (Uekama et al., 1982; Bently et al., 1997; Vianna et al., 1998). Other investigators have reported that HPβCD may increase the skin permeation by acting as a carrier of drug molecules through an aqueous diffusion layer (Loftsson et al., 1994; Loftsson and Sigurdardottir, 1994; Vollmer et al., 1994; Masson et al., 1999; Lopez et al., 2000). In light of these reports, further studies were deemed needed to determine whether cyclodextrins themselves are skin permeation enhancers before they can be used as solubilizing agents in mechanistic studies of skin permeation enhancers.

The purpose of the present study was to address the question: does HPβCD enhance skin permeation in any way? Corticosterone (CS) was selected as the model permeant as it has been shown to be particularly suitable for quantitatively probing the lipoidal pathway of the stratum corneum (Yoneto et al., 1995a). Hairless mouse skin (HMS) was chosen as the model skin membrane for this study as it has been shown to represent human skin well for mechanistic skin permeation enhancer investigations (Kim et al., 1992b; Yoneto et al., 1995a,b) and also provides results of reasonably good reproducibility. While the main focus of this study was to determine the possible role(s) of HPβCD on skin permeation enhancement, the effects of added polyvinylpyrrolidone (PVP) and that of autoclaving the CS suspensions at 121 °C prior to the permeation experiments are included in this study as these variables were routinely part of the studies of Loftsson et al. who are authors of several key reports on this subject (Loftsson et al., 1994; Loftsson and Sigurdardottir, 1994; Masson et al., 1999).

2. Materials and methods

2.1. Materials

[^{3}H]Corticosterone (^{3}H-CS) at >95% purity was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO) and tested for purity by methods suggested by the supplier. Phosphate buffered saline (PBS) tablets, pH 7.4, were obtained from Sigma Chemical Co. (St. Louis, MO) and used as received. Non-radiolabeled corticosterone (Sigma Chemical Co.) was recrystallized in 100% ethanol. Hydroxypropyl-β-cyclodextrin (HPβCD; MW ∼ 1380, MS = 0.6) and polyvinylpyrrolidone (PVP; MW ∼ 360,000) were purchased from Sigma. Cellulose ester membrane (Spectra/por® MWCO 500) was obtained from Spectrum Laboratories Inc. (Rancho Domingue, CA). Female hairless mice (strain SKH-HRI, 8–12 weeks old) were obtained from Charles River (Wilmington, MA).

2.2. Solubility experiments

Exactly 70 mg of corticosterone (CS) previously recrystallized in 100% ethanol was added to 8 ml of

solutions of different concentrations of HPβCD in PBS, in screw capped Pyrex culture tubes (diameter, 13 mm; length, 100 mm; VWR Scientific, Philadelphia, PA). The tubes were shaken for 96 h at 37 °C in a thermostatted water bath (model YB-521, American Scientific Products, McGaw Park, IL). The tubes were then centrifuged (Cole Parmer, Vernon Hills, IL) for 15 min at 3500 rpm (1318 × g). The clear supernatant solution was filtered through Millipore® filter (type GS, 0.22 μm pore size, 25 mm diameter) previously saturated with the CS solution, and analyzed for CS concentration by HPLC. The HPLC system consisted of a HP 1100 series pump and a HP 1050 series auto-sampler with a variable wavelength UV absorbance detector and a 15 cm × 4.6 mm, packing particle size 4.0–4.3 μm Discovery® C18 column (Supelco, Bellefonte, PA). Methanol/water ratio of 66:34 was used as the mobile phase at a flow rate of 1.0 ml/min. Another set of tubes was checked for CS solubility after 15 days. A phase–solubility diagram is constructed by plotting the total molar concentration of CS on the y-axis and the total molar concentration of cyclodextrin added on the x-axis. The stability constant (K_c) of CS/HPβCD complexes was determined with the assumption of a one-to-one stoichiometry. This assumption will be discussed and justified later in Sections 3.1 and 3.2. From the slope of phase–solubility diagram and the solubility of CS in PBS (So), K_c was estimated according to Eq. (1) (Higuchi and Connors, 1965):

$$K_c = \frac{S_t - S_0}{S_0(L_t - (S_t - S_0))} \quad (1)$$

where S_0 is the molar intrinsic CS solubility, S_t is the total molar CS concentration in presence of total molar HPβCD concentration (L_t). This K_c value was later used for calculating the free CS concentration in the donor chamber.

2.3. Silicone polymer partitioning experiments

As the K_c value determined by Eq. (1) is only valid for saturated solutions, another method was used to determine K_c at low CS concentrations (down to trace levels). This method is based on the ^{3}H-CS PBS/silicone polymer partitioning. Sheets of silicone were prepared by mixing equal amounts of dimethylsiloxane elastomers (Nusil, Silicone Technology, Carpinterie, CA) under vacuum. The mixture was spread onto glass dishes and allowed to cure at 150 °C for 10 min then left at room temperature for 24 h prior to use. Discs of 7 mm diameter and 1 mm thickness were cut and weighed. The discs were placed in 2 ml vials and equilibrated with 1 ml solutions of various CS concentrations with or without ^{3}H-CS added in PBS from trace levels to CS solubility and with different concentrations of HPβCD present. The vials were left for 1–8 days to equilibrate in a shaking water bath at 37 °C. There was no significant difference between the partitioning data determined in the experiments with 2–8 days (data not shown). Therefore, all experiments were carried out by leaving the vials for 4 days at 37 °C. After equilibrium was attained, aliquots from the aqueous phase were withdrawn and analyzed for ^{3}H-CS content by scintillation counter and for non-radiolabeled CS content by HPLC. The ^{3}H-CS or CS content of the silicone discs was also determined after double extraction with absolute ethanol. The CS silicone/aqueous partition coefficients in different HPβCD concentrations were determined and used to calculate the complexation binding constant from Eq. (2):

$$K_c = \left[\frac{(K_0/K_x) - 1}{L_f}\right] \quad (2)$$

where, K_0 is CS partition coefficient in silicone/PBS system, K_x is the CS partition coefficient in silicone/aqueous HPβCD system, K_c (as in Eq. (1)) is the stability constant of the complex and L_f is the free molar HPβCD concentration in aqueous phase. For partitioning experiments in which only ^{3}H-CS was used $L_f \approx L_t$.

2.4. Permeation through cellulose ester membrane to examine the effects of PVP, PVP/HPβCD and autoclaving at 121 °C

Excess amount of CS was added to aqueous solutions containing 0.25 and 1% PVP with or without 5% HPβCD. Some of the suspensions were directly equilibrated at 37 °C for 3 days, and others were first heated in an autoclave to 121 °C for 20 min, and then allowed to equilibrate for at least 3 days at room temperature. After equilibration, suspensions were filtered through 0.22 μm filter previously saturated with CS solution, and analyzed for CS concentration by HPLC. The PVP

and PVP/HPβCD supernatants were used in studies of CS transport across seven cellulose ester membranes MWCO 500 after equilibrating the supernatants with added ^{3}H-CS. The permeability experiments were carried out at 37 °C using a two-chamber side-by-side diffusion cell system (effective diffusional area, 0.78 cm^2), with the cellulose ester membrane sandwiched between the two half-cells, each having a 2-ml volume. The fluxes and the apparent permeability coefficients of both non-radiolabeled CS and ^{3}H-CS were calculated from the experimental data.

2.5. Hairless mouse skin permeability experiments

The permeability experiments with hairless mouse skin were carried out at 37 °C using a two-chamber, side-by-side diffusion cell system with each compartment having a 2-ml volume and an effective diffusional area 0.78 cm^2 (Ghanem et al., 1992; Kim et al., 1992a). The experiments were conducted with full-thickness hairless mouse skin, freshly obtained from the abdomen region and freed from adhering fat and other visceral debris. The skin membrane was first checked to ensure that no visual defects were present; then it was sandwiched between the two half cells and 2 ml of HPβCD solution in PBS was pipetted into both chambers with stirring (≈150 rpm). To achieve equilibrium of the HPβCD solution with the skin membrane, the system was left under this condition for 3 h. After equilibration, ^{3}H-CS was added to the donor chamber in each of three different configurations: (1) symmetric configuration with HPβCD present at equal concentrations in both the donor and the receiver chambers; (2) asymmetric, with HPβCD in only the donor chamber; (3) symmetric, with HPβCD in both the donor and the receiver chambers and with saturated CS in PBS in the donor chamber. At predetermined time intervals, 10 μl aliquots from the donor chamber and 500 μl aliquots from the receiver chamber were taken, mixed with scintillation cocktail (Ultima Gold, Packard Instrument Co., Meriden, CT) and analyzed with a liquid scintillation counter (Packard TriCarb Model 1900TR Liquid Scintillation analyzer). After each time a sample was taken, the same volume of fresh HPβCD solution or PBS (according to system configuration) was added back to the receiver chamber to maintain a constant solution volume. The experimental apparent permeability coefficient (P_{exp}) for the permeant was then calculated according to Eq. (3):

$$P_{exp} = \frac{1}{AC_D}\frac{dQ}{dt} \quad (3)$$

where, A is the diffusional area of the diffusion cell, C_D is the total permeant concentration in the donor chamber, dQ/dt is the slope in the steady-state region of the amount of permeant (Q) transported into the receiver chamber versus time (t) plot. Experiments were always run long enough so that the steady-state region was typically around three to five times longer than the lag time.

2.6. The effect of PVP on CS permeability through hairless mouse skin

The transport experiments were conducted with hairless mouse skin with the same PVP and PVP/HPβCD supernatants used in transport studies with the cellulose ester membrane. The effects of PVP and PVP/HPβCD on the permeability coefficient and the flux of ^{3}H-CS were determined and compared to the results obtained from cellulose ester membrane experiments.

2.7. Detection of HPβCD in the receiver chamber

A colorimetric technique was used to detect the presence of the HPβCD in the receiver chamber (Makela et al., 1987). The permeability experiments were carried out at 37 °C using two-chamber, side-by-side diffusion cell system with the donor chamber having 2 ml 5% HPβCD solution in PBS. After 14 h, the HPβCD content in both donor and receiver chambers was determined. The 2 ml solution in each chamber was prepared for analysis by adding 500 μl of 1.0 M Na_2CO_3 solution, followed by 200 μl of phenolphthalein standard solution 1 mg/ml in ethanol (Sigma Diagnostics, St. Louis, HO). The volume was completed to 5 ml with PBS. In the control assays an equal volume of PBS replaced the sample. The color intensity was immediately measured at 550 nm against the blank at 25 °C by UV-visible spectrometry (Uvikon 810, Kontron Instruments, CA). The HPβCD concentration was calculated from a calibration curve made by using 1–2000 μl of the HPβCD standard.

2.8. Reversibility studies

Diffusion cells were assembled with full thickness HMS as described earlier for a typical permeation experiment. However in this protocol, 2 ml of 1% or 5% HPβCD solution was pipetted into both chambers, and then replaced several times with fresh solution over a period of 3 h at 37 °C with stirring (150 rpm). Both chambers of the diffusion cell were then rinsed several times with PBS. Following the PBS rinsing, transport experiments with ^{3}H-CS were carried out with PBS in both chambers. The HMS permeability coefficients obtained with PBS after HPβCD solution pretreatment were compared with permeability coefficients obtained without the HPβCD pretreatment.

2.9. Physical model approach in skin permeation data analysis

The method and procedure for data analysis with the transport model have been previously described (Ghanem et al., 1987; Kim et al., 1992b; Yoneto et al., 1995a). The pertinent equations employed in the data analysis are briefly reviewed here. The experimental permeability coefficient (P_{exp}) for full-thickness HMS may be expressed:

$$P_{exp} = \frac{1}{(1/P_{SC}) + (1/P_{D/E})} \tag{4}$$

Here, P_{SC} is the permeability coefficient for the stratum corneum and $P_{D/E}$ is the permeability coefficient for the dermis–epidermis combination and can be estimated from permeation experiments with tape-stripped skin. P_{SC} can be further divided into parallel lipoidal and pore pathway components in the stratum corneum via the following equation:

$$P_{SC} = P_L + P_P \tag{5}$$

where P_L and P_P are the permeability coefficients for the lipoidal and pore pathways, respectively. Substituting Eq. (5) into Eq. (4) yields the expression:

$$P_{exp} = \frac{1}{(1/(P_L + P_P)) + (1/P_{D/E})} \tag{6}$$

Based on results from a previous study (Yoneto et al., 1995a), we have selected the non-labeled CS or ^{3}H-CS as the probe permeant because corticosterone, most conveniently, allows Eq. (6) to be well approximated by:

$$P_{exp} \approx P_L \tag{7}$$

In an analysis of experimental data obtained with HMS in PBS, Eq. (7) has been found to be true, viz., that, for corticosterone in PBS, $P_P \ll P_L$ and $P_{D/E} \gg P_L$ (Warner et al., 2001). Eq. (7) can then be used to approximate P_L values in the corticosterone transport studies up to enhancement factors of $\leq$10. For greater enhancements, Eq. (6) should be used with estimated values for $P_{D/E}$.

As P_{exp} in Eqs. (6) and (7) is based on total permeant concentration, C_D (see Eq. (3)), it is useful to have an expression for $P_{exp,f}$, the permeability coefficient based upon the free CS concentration in the donor chamber:

$$P_{exp,f} = P_{exp}[1 + K_c(L_f)] \tag{8}$$

The expression for the enhancement factor, E, may then be written as:

$$E = \left(\frac{P_{exp,f}}{P_{exp,PBS}}\right) \tag{9}$$

where $P_{exp,PBS}$ is the experimental permeability coefficient in PBS.

3. Results

3.1. Solubility data

Steroids exhibit widespread polymorphism that may contribute to variable solubility behavior (Byrn, 1982). Previous studies from our laboratory (Warner et al., 2001) showed that recrystallization of CS in 100% ethanol resulted in constant CS solubilities with crystal excesses up to 100 times the solubility. This recrystallized CS was used in our solubility studies. Fig. 1 presents the solubility behavior of CS as a function of HPβCD concentration in PBS. At HPβCD concentrations below 5% (w/v) the solubility diagram was found to be of Higuchi's A_L-type, i.e., linear increase was observed with unchanged stoichiometry (Higuchi and Connors, 1965). The linear A_L-type diagram also suggests CS/HPβCD is predominantly a one-to-one complex in nature within the range of the HPβCD concentration studied. This conclusion is also supported

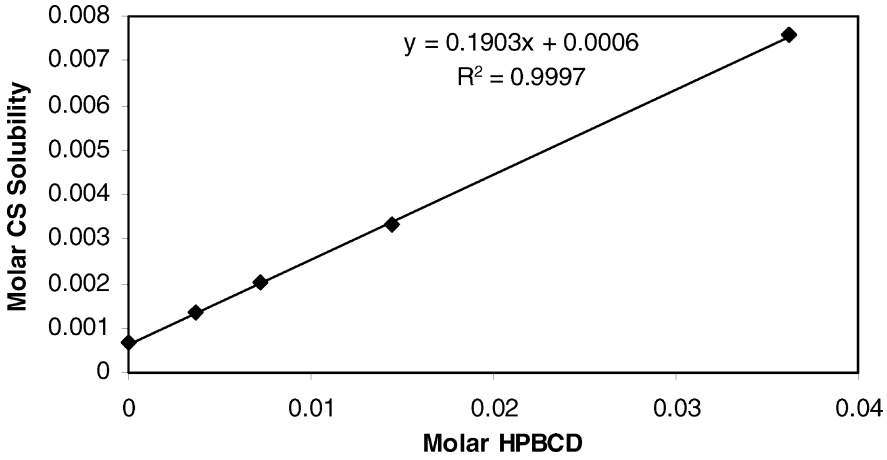

Fig. 1. Phase–solubility diagram of corticosterone in HPβCD solution.

by the results in Section 3.2. The complexation binding constant was therefore estimated from the slope of phase–solubility diagram using Eq. (1). The binding constant determined by this method from the data of Fig. 1 was $350 \pm 7\,M^{-1}$.

3.2. Silicone polymer partitioning experiments

As the K_c value determined by Eq. (1) is based on CS saturated solution data only, another method was needed to determine the K_c value at lower CS solution concentrations. The method selected involves ^{3}H-CS partitioning from the aqueous phase into silicone polymer and the treatment of the data using Eq. (2). There was no measurable depletion of HPβCD in the system due to the uptake of HPβCD into silicone within the sensitivity limit of the colorimetric assay. Table 1 shows the values of the complexation binding constant (K_c) obtained at saturation and at trace levels in 1 and 5% HPβCD with the silicone polymer partitioning method. The results show that all the data are consistent with a one-to-one complex governing the equilibrium behavior from trace levels to saturated solutions. The K_c values calculated from the silicone partitioning method ($357–369\,M^{-1}$) are also consistent with the value obtained from the solubility data ($\sim 350\,M^{-1}$). This shows the appropriateness of using a single binding constant ($K_c \approx 360\,M^{-1}$) for the inclusion complex from trace levels of CS to saturation in calculating the free CS concentration in the donor chamber of the transport experiments.

Table 1
Determination of K_c at saturation by the solubility method and at trace levels by the silicone polymer partitioning method[a]

	Cyclodextrin concentration (% w/v)	K_c values (M^{-1})
Corticosterone saturated solution	1	373.4 ± 18 ($n = 9$)
	5	367.8 ± 15 ($n = 8$)
Trace level corticosterone	1	357.1 ± 12 ($n > 6$)
	5	369.1 ± 20 ($n > 6$)

[a] K_c determined from the solubility data and Eq. (1) was $350\,M^{-1}$.

3.3. Permeation through cellulose ester membrane to examine the effects of PVP, PVP/HPβCD and autoclaving at 121 °C

The results of the transport experiments with the cellulose membrane are presented in Table 2. First, it is seen that CS fluxes based on the ^{3}H-CS measurements (column 3) and those based on the direct determinations of the non-radiolabeled CS fluxes by HPLC (column 7) are the same within experimental error in each situation. This means that proper equilibrium had been attained between ^{3}H-CS and the non-radiolabeled CS. While this would seem to be an obvious outcome, this test of equilibrium should be considered necessary when supersaturated systems are involved. The P_{exp} values (columns 4 and 8) and the R

Table 2
Determination of the effects of PVP, PVP/ HPβCD, and autoclaving at 121 °C on the transport of ^{3}H-CS and non-radiolabeled CS across semi-permeable cellulose membrane from saturated and supersaturated CS solutions

Condition of the experiment ^{3}H-CS equilibrated with saturated or supersaturated solution of CS in:	Solubility and CS donor concentration (mg/ml)	^{3}H-CS				Non-radiolabeled CS			
		Flux[a] (S.D.) (mg/min/cm^2 × 10^4)	P_{exp} (S.D.) (cm/s × 10^5)	$P_{exp.f}$ (cm/s × 10^5)	R[b]	Flux[c] (S.D.) (mg/min/cm^2 × 10^4)	P_{exp} (S.D.) (cm/s × 10^5)	$P_{exp.f}$ (cm/s × 10^5)	R[b]
PBS at 37 °C	0.23	3.7 (0.16)	2.6 (0.14)	2.6	–	3.5 (0.3)	2.56 (0.22)	2.6	–
0.25% PVP/ PBS at 37 °C	0.23	3.8 (0.10)	2.88 (0.13)	2.9	1.03	3.8 (0.0)	2.78 (0.0)	2.8	1.08
0.25% PVP/ PBS at 37 °C after 121 °C treatment	0.78	12.2 (0.15)	2.62 (0.04)	2.6	3.3	13.0 (0.06)	2.87 (0.14)	2.9	3.7
5% HPβCD/ PBS at 37 °C	2.36	3.8 (0.18)	0.27 (0.17)	2.8	1.03	3.84 (0.0)	0.27 (0.0)	2.8	1.09
1% PVP/ PBS at 37 °C	0.23	3.8 (0.05)	2.76 (0.03)	2.8	1.03	3.8 (0.0)	2.78 (0.0)	2.8	1.08
1% PVP/PBS at 37 °C after 121 °C treatment	0.79	12.8 (0.0)	2.72 (0.05)	2.72 (0.05)	3.46	13.0 (0.1)	2.80 (0.1)	2.8	3.71
5% HPβCD + 0.25% PVP/PBS at 37 °C	2.4	3.9 (0.13)	0.27 (0.13)	2.8	1.05	4.1 (0.3)	0.28 (0.16)	2.9	1.17
5% HPβCD + 0.25% PVP/PBS at 37 °C after 121 °C treatment	7.8	13.4 (0.01)	0.28 (0.03)	2.9	3.62	14.1 (0.0)	0.30 (0.0)	3.1	4.02

[a] The flux of non-radiolabeled CS calculated from the flux of ^{3}H-CS.
[b] R is the ratio of CS flux of the corresponding system to CS flux of PBS at 37 °C (control).
[c] The flux of non-radiolabeled CS as measured by HPLC.

values (columns 6 and 10) are accordingly seen to be essentially the same whether they are based on ^{3}H-CS or on the non-radiolabeled CS measurements. When the solution systems were not autoclaved at 121 °C, the flux ratio, *R*, (columns 6 and 10) remained close to unity in every case. This result was as expected since (a) at thermodynamic equilibrium, the free CS concentration should be the same in all systems; and (b) with a molecular weight cut-off of 500, only the free CS was expected to be transported across the cellulose membrane. Neither HPβCD (MW ∼ 1380) nor the CS/HPβCD complex would have been expected to be able to permeate the small pores of the cellulose membrane at an appreciable rate.

In contrast to these results, it is seen that when the systems were autoclaved at 121 °C with PVP present (rows 3, 6, and, 8) the flux ratio in each of the three cases shown in Table 2 is seen to be significantly greater than unity and in the range of 3–4. It is difficult to suggest any other interpretation of this result than that the free CS concentration in these three cases were three to four times greater than that for CS solubility in PBS at 37 °C (column 2). To have a supersaturated state (with a supersaturation ratio of 3–4) in this situation, however, would not be surprising. There is much precedence that supersaturation ratios as high as 15 times the solubility may be sustained for steroids in the presence of PVP (Simonelli et al., 1970; Corrigan et al., 1980). Also, preheating steroid suspensions at 121 °C should easily result in higher free CS concentration as the temperature dependence of aqueous solubilities of organic compounds are generally positive (see, e.g. Grant and Higuchi, 1990). As the temperature is allowed to drop back to 37 °C, PVP, which is a well-known nucleation and crystal growth inhibitor (Simonelli et al., 1970; Corrigan et al., 1980), can sustain the supersaturated state for long periods of time. The autoclaving results with 0.25% PVP and 1% PVP are seen to be essentially the same. This is consistent with the finding of Simonelli et al. (1970) that crystal growth inhibition by PVP can be independent of PVP concentration at low to moderate supersaturations.

There are several other important aspects of the results presented in Table 2. As PVP at either 0.25% or 1% is seen not to alter the CS solubility (rows 2 and 4), one may conclude that there cannot be any significant interaction between PVP and CS in solution at these levels. Also, the presence of PVP at 0.25% level does

Table 3
CS solubility and permeability coefficient of ^{3}H-CS at different concentrations of HPβCD with hairless mouse skin (symmetric configuration)

HPβCD concentration (% w/v)	CS solubility ratio[a]	Apparent permeability coefficient (P_{exp}) of ^{3}H-CS in saturated solution[b] (cm/s)	Apparent permeability coefficient (P_{exp}) of ^{3}H-CS at trace levels[b] (cm/s)	Permeability coefficient calculated on the basis of free CS ($P_{exp,f}$)[c] (cm/s)
0		$(2.2 \pm 0.3) \times 10^{-7}$	$(2.2 \pm 0.3) \times 10^{-7}$	
1	3.0 ± 0.01	$(7.0 \pm 0.7) \times 10^{-8}$	$(7.2 \pm 0.8) \times 10^{-8}$	$(2.31 \pm 0.3) \times 10^{-7}$
5	10.2 ± 0.2	$(2.4 \pm 0.2) \times 10^{-8}$	$(2.3 \pm 0.3) \times 10^{-8}$	$(2.16 \pm 0.4) \times 10^{-7}$

[a] Solubility ratio = (solubility in HPβCD solution)/(solubility in PBS).
[b] The experimental permeability coefficient calculated by Eq. (3).
[c] Permeability coefficient calculated by Eq. (8) that corrects for free CS in donor chamber on the basis of CS/HPβCD binding constant.

not alter CS solubility in 5% HPβCD (row 7); therefore, there cannot be any interaction or interference between PVP and HPβCD, significantly influencing the ability of HPβCD to solubilize CS. The absence of the effect of PVP upon CS solubility should permit one to predict that: (a) $P_{exp,f} = P_{exp}$ when only PVP is present; and (b) $P_{exp,f} = P_{exp}[1 + K_c(L_f)]$ when HPβCD is present with or without PVP. That this prediction is true can be seen in columns 5 and 9 of Table 2.[1] This outcome with the synthetic cellulose membrane is important as Eq. (8) or Eq. (9) may now be used with a high degree of confidence in addressing the question of whether HPβCD, alone or together with PVP, may act as a permeation enhancer for CS transport across the HMS membrane.

3.4. CS HMS transport experiments with CS solubilized in HPβCD solutions

The results of all the HMS transport experiments with HPβCD conducted with the symmetric configuration (i.e. HPβCD present in donor as well as in receiver chamber) with ^{3}H-CS as the permeant are presented in Table 3. The decrease in experimental apparent permeability coefficient P_{exp} (columns 3 and 4) is due to formation of the drug–cyclodextrin inclusion complex that lowers the free drug concentration (and reduces the drug thermodynamic activity). The permeability coefficient for CS in HPβCD calculated on the basis of the free drug concentration ($P_{exp,f}$) by Eq. (8), is presented in column 5 of Table 3. The data show that the $P_{exp,f}$ values of CS in HPβCD solution were relatively constant and also independent of the HPβCD concentration. These results are direct evidence that HPβCD does not function as a HMS permeation enhancer for CS. The results of transport experiments that were conducted with HPβCD in the donor cell chamber only (asymmetric configuration) are presented in Table 4. It was found that there is no significant difference between the permeability coefficients of CS obtained from the symmetric and asymmetric configurations.

3.5. The effect of PVP and of PVP with autoclaving on CS transport across HMS

The results of ^{3}H-CS transport experiments with 0.25 and 1% w/v PVP in CS saturated solutions with and without autoclave treatments are presented in Table 5. The fluxes of ^{3}H-CS over a wide range of conditions and the R values are presented in columns 3 and 6, respectively. All results parallel the results obtained in the cellulose membrane experiments (see Table 2). The 3–4-fold increase in the CS flux from autoclaved PVP solutions with or without cyclodextrin is a result of CS supersaturation. Importantly, the $P_{exp,f}$ values in column 5 are all seen to be essentially the same. This can be only interpreted as that neither HPβCD alone nor together with PVP is able to act as a HMS permeation enhancer for CS.

3.6. Detection of cyclodextrin in the receiver chamber

The sensitivity of the colorimetric technique that was used to detect the presence of HPβCD in the

[1] The physical meaning of $P_{exp,f}$, as applied here in the context of the cellulose membrane differs from that for $P_{exp,f}$ based on Eqs. (6)–(8) for HMS. $P_{exp,f}$ in Eq. (8) in the case of the HMS membrane is for the lipoidal pathway of the HMS SC while, for the case of the cellulose membrane, $P_{exp,f}$ refers to the aqueous pore pathway of the synthetic membrane. However, the same Eq. (8) may be employed in both cases.

Table 4
Apparent permeability coefficient of corticosterone in HPβCD (symmetric and asymmetric configurations)

HPβCD concentration (% w/v)	Apparent permeability coefficient (P_{exp}) of ^{3}H-CS (symmetric)[a] (cm/s)	Apparent permeability coefficient (P_{exp}) of ^{3}H-CS (asymmetric)[b] (cm/s)
0	$(2.2 \pm 0.3) \times 10^{-7}$	$(2.2 \pm 0.3) \times 10^{-7}$
1	$(7.2 \pm 0.8) \times 10^{-8}$	$(6.9 \pm 0.5) \times 10^{-8}$
5	$(2.3 \pm 0.3) \times 10^{-8}$	$(2.2 \pm 0.4) \times 10^{-8}$

[a] From Table 3.
[b] Permeability coefficient calculated by Eq. (3).

Table 5
Determination of the effects of PVP, PVP/HPβCD, and autoclaving at 121 °C on the HMS membrane transport of ^{3}H-CS in CS saturated solutions

Condition of the experiment, ^{3}H-CS equilibrated with CS saturated or supersaturated solution of CS in:	S[a] (mg/ml)	Flux (mg/h/cm^2 × 10^4)	P_{exp} (S.D.) (cm/s × 10^7)	$P_{exp,f}$ (S.D.) (cm/s × 10^7)	R[b]
PBS at 37 °C	0.23	1.8 (0.2)	2.2 (0.3)	2.2	1.0
0.25% PVP/PBS at 37 °C	0.23	2.0 (0.1)	2.4 (0.2)	2.4	1.1
0.25% PVP/PBS at 37 °C after 121 °C treatment	0.78[c]	6.5 (0.2)	2.3 (0.2)	2.3	3.6
5% HPβCD at 37 °C	2.36	2.0 (0.4)	0.23 (0.3)	2.4	1.1
1% PVP/PBS at 37 °C	0.23	1.8 (0.2)	2.2 (0.1)	2.2	1.0
1% PVP/PBS at 37 °C after 121 °C treatment	0.79[c]	6.8 (0.3)	2.4 (0.3)	2.4	3.8
5% HPβCD + 0.25% PVP/PBS at 37 °C	2.4	1.9 (0.3)	0.22 (0.2)	2.3	1.1
5% HPβCD + 0.25% PVP/PBS at 37 °C after 121 °C treatment	7.8[c]	6.7 (0.2)	0.24 (0.1)	2.5	3.7

[a] S is the CS solubility unless otherwise indicated.
[b] R is the ratio of CS flux of the corresponding system to CS flux of PBS (37 °C).
[c] CS supersaturated solutions.

receiver chamber was 4.24×10^{-6} M. The assay revealed no HPβCD in the receiver chamber, at the end of the 8-h CS transport experiments under the asymmetric configuration. At the same time, analysis of the donor chamber showed no change in HPβCD concentration. This suggests that HPβCD and the CS/HPβCD complex do not effectively partition into or transport across the skin. It has been reported that ^{14}C beta-cyclodextrin (βCD) was not absorbed in its intact form either in the stomach or the small intestine of rat (Okamoto et al., 1986) (i.e., HPβCD does not penetrate a membrane that is expected to be more permeable than skin), supporting that skin is impermeable to cyclodextrin derivatives and/or their complexes.

3.7. Reversibility studies

Table 6 presents the results of the reversibility studies. The permeability coefficients of HMS obtained in PBS after HMS pretreatment with the HPβCD solution were the same as those obtained without cyclodextrin pretreatment. This supports that HPβCD did not extract lipids or membrane components to any significant extent. There was no evidence that the barrier function of the skin was altered as suggested by some investigators (Uekama et al., 1982; Bently et al., 1997; Vianna et al., 1998).

Table 6
Reversibility after pretreatment of hairless mouse skin with HPβCD solutions

Pretreatment HPβCD concentration (% w/v)	Permeability coefficient of CS after pretreatment (cm/s × 10^7)	R[a]
0	2.2 ± 0.3	–
1	1.9 ± 0.4	0.86 ± 0.2
5	2.1 ± 0.2	0.95 ± 0.2

[a] R is the ratio of (P_{exp} value, after pretreatment)/(P_{exp} value, no pretreatment).

4. Discussion

The results of transport experiments with the synthetic cellulose membrane and with HMS demonstrate that HPβCD, PVP, or the PVP/HPβCD combination does not enhance the flux of CS in any way in either of the two membranes studied. The role of HPβCD is only that of a solubilizing agent for CS. For the conditions of the present study, PVP does not interact with CS nor with HPβCD in solution in any significant manner. It is seen that the $P_{exp,f}$ values for HMS (Tables 3–5) remain essentially constant for all situations. These results are interpreted to mean that the barrier properties of HMS stratum corneum are not altered under the conditions of these experiments. There is also no evidence of significant relevant lipid extraction (Table 6) or altered state of relevant lipid fluidization as, otherwise, the $P_{exp,f}$ values would not have remained constant in these studies. The constant $P_{exp,f}$ values of Tables 3 and 5 also demonstrate the absence of any significant "carrier" effect of HPβCD to enhance CS transport across the HMS membrane. Were a carrier effect important, higher values of P_{exp} and $P_{exp,f}$ would have been found in the cases where HPβCD was involved. Finally, for the same reason, there is also no evidence of an aqueous boundary layer contributing to the transport kinetics: if an aqueous boundary layer contribution were to have been important, higher apparent P_{exp} and higher apparent $P_{exp,f}$ values would have resulted in those experiments (Tables 3 and 5) involving HPβCD, and this is seen not to be the case.

As a main reason for the present study was the literature reports that cyclodextrin derivatives may function as skin permeation enhancers, it is now worthwhile to review some of these reports in light of our findings. In one report, Loftsson et al. (1994) suggested a mechanism that HPβCD keeps the drug molecules in solution and delivers them to the surface of the barrier where they partition into and then through the barrier. In the context of our experiments, this mechanism would be an aqueous boundary layer effect lessened by the CS/HPβCD complex assisting transport of CS across the aqueous boundary to the skin surface. Our studies showed no indication of a significant aqueous boundary layer contribution to CS transport resistance. In another investigation from their laboratory (Loftsson and Sigurdardottir, 1994) on the effect of PVP and HPβCD on the flux of hydrocortisone from autoclaved solutions through hairless mouse skin in vitro, the authors reported that the addition of PVP increased the flux of hydrocortisone from suspensions in the presence of HPβCD. They suggested the increased flux is related to an increased hydrocortisone–cyclodextrin complexation binding constant. They also suggested that PVP may act as a co-enhancer for the cyclodextrin enhanced transdermal delivery of hydrocortisone. In our studies, complexation with HPβCD only decreased the thermodynamic activity of CS and increased flux was never observed. There was also no evidence in our studies that HPβCD, PVP, or the combination acted as a permeation enhancer for CS. Loftsson et al. also did not consider the possibility of supersaturation as a factor in their studies. In our studies autoclaving resulted in a 3–4-fold increase in the free CS concentration; this supersaturated state was stabilized by PVP. Corrigan et al. (1980) have shown that hydrocortisone supersaturation ratios as high as 15-fold may be sustained by PVP.

Possible reasons for the apparent discrepancy between our results and those of Loftsson et al. may include the following. Loftsson et al. (1994) used full-thickness hairless mouse skin in their permeation studies over a 2-day period. This raises the question of the quality of the data when such long experimental times are involved; a previous study involving HMS from our laboratory (Lambert et al., 1989) showed dramatic increases in permeability coefficients following long-term hydration. Another factor that might be responsible for the discrepancy between our results and those of Loftsson et al. is the difference in the diffusion cell and stirring (or the lack there of) in the donor chamber. Ours was a side-by-side horizontal well-stirred system while Loftsson et al. employed a Franz diffusion cell with no stirring in the donor compartment. In our well-stirred system, the aqueous boundary layer has a negligible influence on the CS flux. In their study, there was no stirring in the donor compartment possibly permitting HPβCD to act as a carrier of the permeant through the aqueous boundary layer. Without having factored out the possible importance of supersaturation, however, it would be difficult to assess the real importance of the carrier effect in the studies of Loftsson et al.

A mechanism similar to that reported by Loftsson et al. was suggested by Lopez et al. (2000). In their

Franz diffusion cell study, they suggested that cyclodextrin complexation may promote the availability of the drug at the skin surface and they also suggested that flux enhancement may arise from extraction of lipids from the stratum corneum. Other investigators have also reported that HPβCD may extract lipids from the stratum corneum, this causing increased permeation of drugs across skin (Bently et al., 1997; Vianna et al., 1998). In our studies, there has been no evidence that lipid extraction by HPβCD is of any significance to CS transport across hairless mouse skin.

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