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RESEARCH PAPER

Influence of Hydroxypropyl-β-cyclodextrin on the Transdermal Permeation and Skin Accumulation of Oxybenzone

Linda A. Felton,* Cody J. Wiley, and Donald A. Godwin

University of New Mexico, Health Sciences Center, College of Pharmacy, 2502 Marble NE, Albuquerque, NM 87131

ABSTRACT

The objective of the present study was to determine the effects of hydroxypropyl-β-cyclodextrin (HPCD) concentration on the transdermal permeation and skin accumulation of a model ultraviolet (UV) absorber, oxybenzone. The concentration of oxybenzone was held constant at 2.67 mg/mL for all formulations, while the HPCD concentrations varied from 0 to 20% (w/w). Complexation of oxybenzone by HPCD was demonstrated by differential scanning calorimetry. A modified Franz cell apparatus was used in the transdermal experiments, with aliquots of the receptor fluid assayed for oxybenzone by high-performance liquid chromatography. From the permeation data, flux of the drug was calculated. Skins were removed from the diffusion cells at specified time points over a 24-hr period and the oxybenzone content in the skin determined. The aqueous solubility of oxybenzone increased linearly with increasing HPCD concentration, following a Higuchi A_L-type complexation. The stability constant of the reaction was calculated from the phase-solubility diagram and found to be 2047 M^{-1}. As the concentration of HPCD was increased from 0 to 10%, transdermal permeation and skin accumulation of oxybenzone increased. Maximum flux occurred at 10% HPCD, where sufficient cyclodextrin was added to completely solubilize

*Corresponding author. Fax: (505) 272-6749; E-mail: lfelton@unm.edu

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all oxybenzone. When the concentration of HPCD was increased to 20%, both transdermal permeation and skin accumulation decreased. These data suggest the formation of a drug reservoir on the surface of the skin.

Key Words: *Complexation; Hydroxypropyl-β-cyclodextrin; Oxybenzone; Reservoir; Skin accumulation; Transdermal permeation*

INTRODUCTION

Due to its accessibility, skin has long been considered a route for systemic drug administration, and research into transdermal drug delivery has expanded greatly over the last several decades.[1,2] The major obstacle to systemic drug absorption is the stratum corneum (SC), the outermost layer of the skin, which is composed of many layers of flat cornified protein-filled cells separated by a unique and complex mixture of lipids.[3] Although many scientists have focused their research efforts towards maximizing systemic absorption of drugs applied to the skin, little attention has been focused on the skin accumulation of compounds.

Accumulation of chemicals in the skin is critical to the performance of sunscreen products. Sunscreens contain ultraviolet (UV) absorbing compounds that filter and absorb solar radiation. To be effective, these chemicals must remain on the surface or in the outermost layers of the skin.[4] A number of commonly used UV absorbers, however, have been shown to rapidly penetrate through the skin and reach the systemic circulation, leaving the skin unprotected against the harmful radiation of the sun.[5–7]

Cyclodextrins are crystalline, cyclic oligosaccharides with a bucket-like structure, having a hydrophobic internal cavity surface and a hydrophilic exterior. This unique shape allows for the formation of inclusion complexes, where a second, lipophilic compound is non-covalently bound within the cavity. Cyclodextrins have been employed in pharmaceutical products to alter the solubility[8–10] and stability [11–13] of various drugs. Recently, researchers have investigated the effects of cyclodextrins on the transdermal permeation of drugs.[14–17] No studies, however, have addressed the influence of cyclodextrins on the accumulation of chemicals in the skin or the potential use of these compounds to enhance the photoprotective effects of sunscreens.

The objective of the current study was to determine the effects of a hydroxypropyl-β-cyclodextrin (HPCD) on the transdermal permeation and skin accumulation of a model UV absorber, oxybenzone. Oxybenzone was expected to complex with HPCD and affect the thermodynamic driving force necessary for dermal penetration.

MATERIALS

2-Hydroxy-4-methoxybenzophenone (oxybenzone), 2-hydroxypropyl-β-cyclodextrin (0.8 molar substitution), aqueous formaldehyde solution (37%), and Brij® 58 [polyoxyethylene (20) cetyl ether] were purchased from Sigma-Aldrich (Milwaukee, WI). Methanol and glacial acetic acid were purchased from Fisher Scientific Company (Fair Lawn, NJ). Chemicals were used as received.

METHODS

Aqueous Solubility of Oxybenzone

The HPCD concentrations investigated in this study included 0, 5, 10, and 20% (w/w). An excess of oxybenzone was added to the deionized water–HPCD solutions. The suspensions were sealed and magnetically stirred for 48 hr. The mixtures were then centrifuged at 3300 rpm for 10 min to separate the undissolved drug. An aliquot of the supernatant was analyzed for oxybenzone content using high-performance liquid chromatography (HPLC).

Differential Scanning Calorimetry

A modulated differential scanning calorimeter (Model 2920, TA Instruments, New Castle, DE) was used to demonstrate HPCD–oxybenzone complexation. Oxybenzone alone, a physical blend of the sunscreen and HPCD, and a dried powder obtained from evaporation of a solution of the two compounds were analyzed for the presence or absence of a melting transition. Samples of approximately 10 mg were sealed in aluminum pans and scanned at a rate of 10°C/min from 10 to 120°C. The modulation signal was set

at 1.592°C/min. The thermograms were analyzed using TA Instruments Universal Analysis software.

Preparation of Oxybenzone–HPCD Formulations

Simple solutions and suspensions of oxybenzone were prepared for the transdermal permeation and skin accumulation studies. The concentration of oxybenzone was 2.67 mg/mL for all formulations. This concentration was slightly less than the maximum aqueous solubility of oxybenzone in a 10% HPCD solution. Thus, formulations containing 0 or 5% HPCD were suspensions, while solutions were formed at higher HPCD concentrations.

In Vitro Transdermal Permeation

The hairless mouse was used as the model for the in vitro transdermal permeability and skin accumulation studies. Six-week-old male SKH-1 hairless mice were obtained from Charles Rivers Laboratories (Wilmington, MA). The animal protocol was approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee. Animals were sacrificed by CO_2 asphyxiation and full-thickness abdominal and dorsal skin was excised. Any extraneous subcutaneous fat was removed from the dermal surface. The skin samples were stored at −10°C (Revco Scientific, Asheville, NC) until utilized. Research involving a variety of skin types, including human, cattle, and nude rat, has demonstrated that freezing prior to experimentation does not alter the transport kinetics of skin.[18,19] At the time of experimentation, skin samples were slowly thawed and mounted onto modified Franz diffusion cells (Permegear, Riegelsville, PA). Each diffusion cell (donor surface area 0.64 cm^2; receptor volume 5.1 mL) contained isotonic phosphate buffer solution (pH 7.2) with 0.1% (v/v) 37% aqueous formaldehyde as a preservative and 0.5% (w/v) Brij® 58 as a solubilizer. The receptor fluid was maintained at 37°C ± 0.5 and continuously stirred at 600 rpm using magnetic stirrers. Following a 1-hr hydration period, 200 μL of the oxybenzone-HPCD formulations were applied to each skin ($n = 4$). Samples (300 μL) of the receptor phase were withdrawn at specified time points over a 24-hr period and stored at −10°C until analyzed. Receptor fluid was immediately replaced with fresh buffer and analysis of samples was corrected for previous UV absorber removed.

In Vitro Skin Accumulation

The skin samples were removed from the Franz diffusion cells at specific intervals over a 24-hr period following application of the drug formulations. Residual drug on the surface of the skin was removed using a cotton tip applicator. Each skin was then briefly rinsed in methanol and patted dry with a lint-free wipe. The skin samples were then weighed, cut into small pieces, placed in 2 mL of methanol, and homogenized using a tissue homogenizer (Biospec Products, Racine, WI). The homogenate was centrifuged for 5 min at 3300 rpm using a Fisher benchtop centrifuge (Pittsburgh, PA). Following centrifugation, 1 mL of the supernatant was removed and stored at −10°C until analyzed using HPLC.

Analytical Method

Analysis of samples was performed using HPLC. The liquid chromatograph consisted of a binary pump solvent delivery system (Model P1500, Thermoseparations Products, Riviera Beach, FL), a 50-μL injection loop autosampler (Model AS 1000, Thermoseparations Products), and a variable-wavelength UV light absorbance detector (Model UV 1000, Thermoseparations Products). The analytical column was a 5-μm pore size, 4.6 mm × 150 mm C_{18} column (Alltech Associates, Inc., Deerfield, IL) with a guard column of the same material. The system was controlled and integrated by a personal computer using chromatography management software (PC 1000, Thermoseparations Products). The detection wavelength was 288 nm. The mobile phase was methanol:water (83:17) containing glacial acetic acid at a concentration of 0.01% (v/v) and the flow rate was 1 mL/min. Retention time for oxybenzone was approximately 6 min.

Data Analysis

Transdermal permeation (μg/cm^2) was determined by analyzing each receptor fluid sample for oxybenzone. Using linear regression, the slope of the linear portion of the permeation–time graph was calculated and multiplied by the concentration of drug applied to the skin to determine flux. Skin accumulation (μg drug/mg skin) was calculated by dividing the amount of drug remaining in the skin by the weight of the skin sample. The mean and standard deviation of

these parameters were calculated. Statistical analysis was carried out using SigmaStat (SPSS Inc., Chicago, IL). For statistical comparison, a one-way analysis of variance test was employed and a Tukey post-test was then used to determine differences between treatment groups. A value of $p < 0.05$ was considered statistically significant.

RESULTS

HPCD–Oxybenzone Complexation

Differential scanning calorimetry was used to demonstrate HPCD–oxybenzone complexation. As shown in Fig. 1, oxybenzone had a melting point of approximately 68.7°C. When the drug was physically blended with HPCD, an endothermic peak corresponding to a melting temperature was observed at 68°C. Powder obtained from solvent evaporation of an aqueous solution of HPCD and oxybenzone showed no melting transition, demonstrating that oxybenzone was complexed by the HPCD. These findings are in agreement with previous research that has shown cyclodextrins to form inclusion complexes with lipophilic compounds.[9,13,20]

Influence of HPCD Complexation on the Aqueous Solubility of Oxybenzone

In the present study, HPCD complexation was found to increase the aqueous solubility of the model UV absorber, as shown in Fig. 2. Oxybenzone was only slightly soluble in water (0.133 mM). The addition of HPCD significantly increased the aqueous solubility of the drug. Furthermore, the increase in oxybenzone solubility was found to be linear, with r^2 value greater than 0.997. These data are in agreement with other researchers who found a linear relationship between drug solubility and HPCD concentration.[8,13]

Based on the linearity of the phase–solubility diagram depicted in Fig. 2, the inclusion of oxybenzone by HPCD was considered a Higuchi A_L-type complexation phenomenon and a 1:1 stoichiometry of the reaction was assumed.[21] The stability constant ($K_{1:1}$) for complexation was estimated using Eq. (1), where S_0 is the intrinsic solubility of oxybenzone:

$$K_{1:1} = \frac{\text{Slope}}{S_0(1 - \text{Slope})} \quad (1)$$

The slope of the line from Fig. 2 was calculated using linear regression and found to be 0.214. Using the intrinsic solubility of oxybenzone as determined from the solubility experiments (0% HPCD), $K_{1:1}$ was found to be 2047 M^{-1}.

Influence of HPCD Complexation on Transdermal Permeation

The transdermal permeation of oxybenzone from the HPCD-containing formulations is shown in Fig. 3. The formulation containing 0% HPCD showed very little penetration through the skin (1.6 μg/cm^2 at

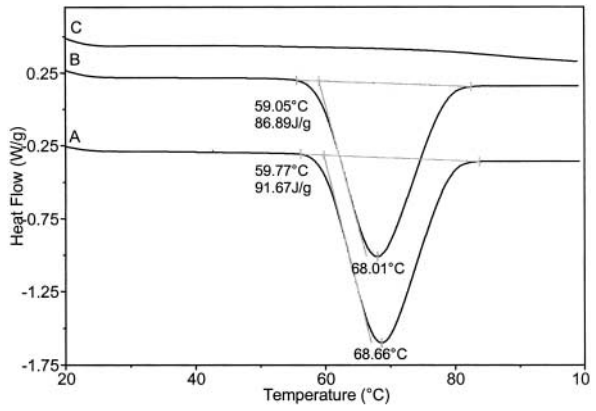

Figure 1. Thermal analysis of (A) oxybenzone alone, (B) a physical blend of oxybenzone and HPCD in the dry state, and (C) oxybenzone and HPCD prepared as a solution and precipitated.

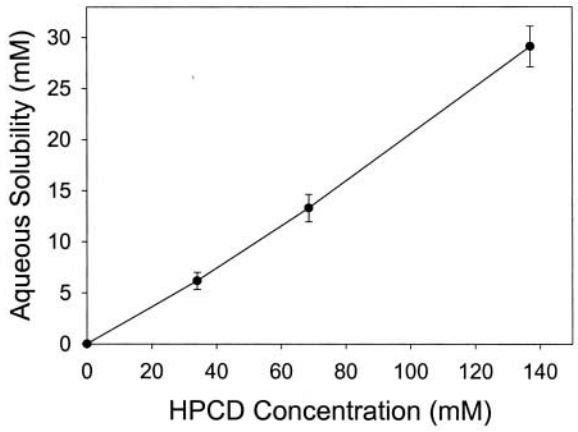

Figure 2. Influence of HPCD concentration on the aqueous solubility of oxybenzone.

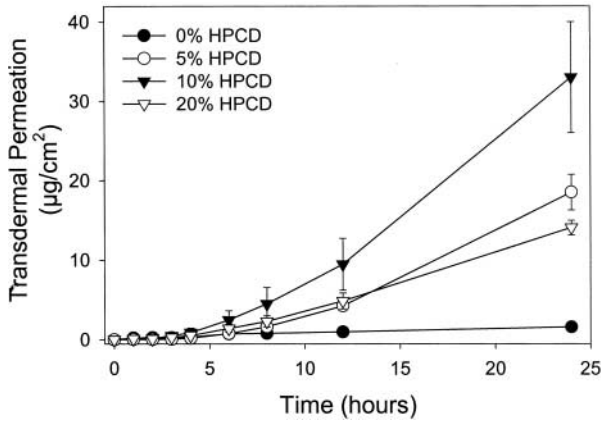

Figure 3. Influence of HPCD concentration on the transdermal permeation of oxybenzone.

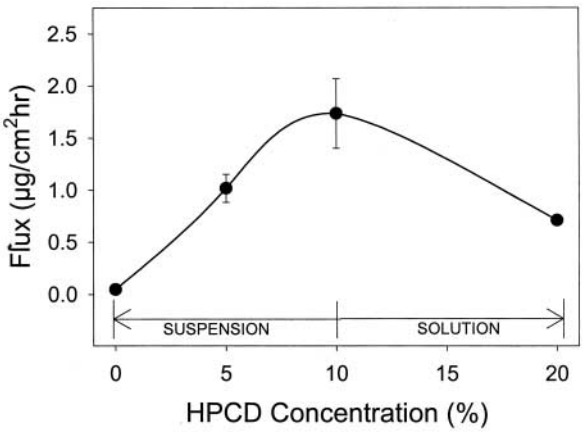

Figure 4. Influence of HPCD concentration on flux of oxybenzone.

24 hr). All formulations containing HPCD exhibited higher transdermal permeation compared to the control. The formulation containing 10% HPCD exhibited the greatest drug permeation (33.01 μg/cm^2 at 24 hr), while permeation through the skin was significantly lower in both the 5 and 20% HPCD formulations (18.53 and 14.08 μg/cm^2 at 24 hr, respectively).

From the transdermal permeation data, the flux of oxybenzone through the hairless mouse skin was calculated and the data are presented in Fig. 4. The flux of oxybenzone from the 0% HPCD formulation was low (0.048 μg/cm^2 hr), and all HPCD-containing formulations showed higher rates of transdermal permeation. The flux of the UV absorber was

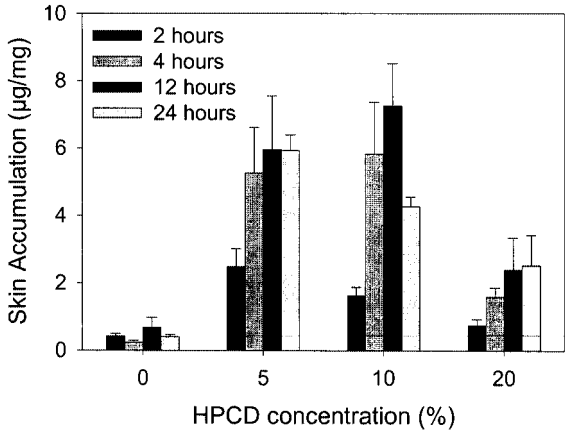

Figure 5. Influence of HPCD concentration on the skin accumulation of oxybenzone.

greatest when applied as a 10% HPCD formulation (1.74 μg/cm^2 hr), whereas permeation rates were lower from both the 5% HPCD suspension (1.02 μg/cm^2 hr) and the 20% HPCD solution (0.71 μg/cm^2 hr). These results are in agreement with Loftsson and coworkers,[22] who showed a similar parabolic relationship between flux and HPCD concentration.

Influence of HPCD Concentration on Skin Accumulation

The amount of drug that accumulated in the skin as a function of HPCD concentration and time post-application is shown in Fig. 5. Oxybenzone skin accumulation was low for the 0% HPCD control formulation (0.40 μg/mg at 24 hr). Significantly higher drug content was found in the skin for all formulations containing HPCD and at all times post-application compared to the control. The 5 and 10% HPCD formulations exhibited the highest oxybenzone skin accumulation (5.93 and 4.26 μg/mg at 24 hr, respectively), while much lower amounts of drug were found in the skin following application with the 20% HPCD formulation (2.52 μg/mg at 24 hr).

DISCUSSION

Previous research has shown that cyclodextrins form inclusion complexes with lipophilic compounds and that these complexes alter various physicochemical properties of drugs.[10–13,20,23]

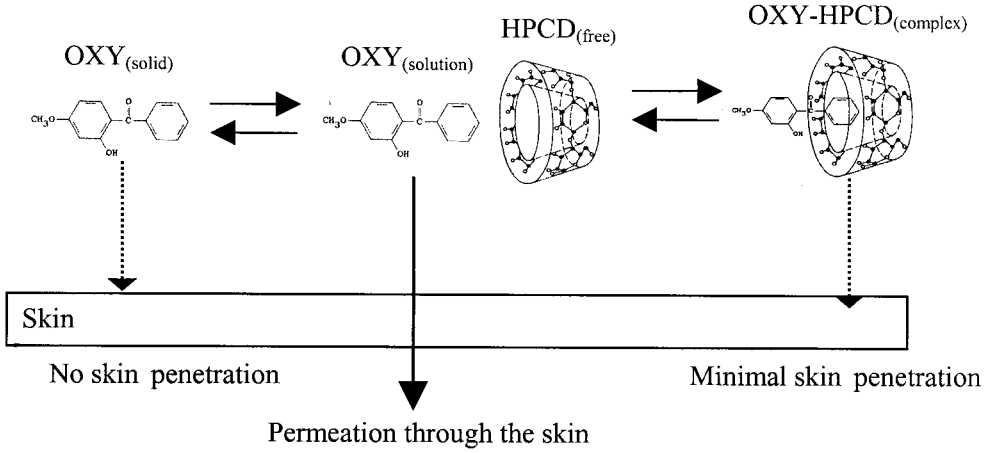

Figure 6. Schematic representation of the complexation and transdermal permeation of the oxybenzone–HPCD system.

Recently, these adjuvants have been added to topical formulations as penetration enhancers.[14,16,24] Cyclodextrins are capable of complexing with lipophilic materials in the stratum corneum,[14] and some researchers have suggested that this interaction alters skin permeability.[25,26] Other researchers, however, have demonstrated that cyclodextrins do not affect the skin barrier.[27] In the current study, increases in transdermal permeation and skin accumulation of oxybenzone were attributed primarily to enhanced drug solubility.

Figure 6 shows a schematic representation of the theoretical complexation and transdermal permeation of oxybenzone. Since drugs must be dissolved in order to penetrate into the skin, solid oxybenzone does not contribute to transdermal permeation. The molecular size and hydrophilic character of the HPCD–oxybenzone complex prevent any significant skin penetration from this species.[28,29] Thus, only oxybenzone in solution permeates through the skin. It is generally believed, however, that hydrophobic drugs are in a hydrated state when complexed and that the cyclodextrins deliver these drugs to the surface of the skin where they partition from the cyclodextrin cavity.

Both flux and skin accumulation of oxybenzone were low for the 0% HPCD suspension, presumably due to very limited aqueous solubility. The addition of 5% HPCD to the formulation resulted in a significant increase in both transdermal permeation and skin accumulation, which was attributed to the higher oxybenzone solubility. When 5% HPCD was added to the formulation, the aqueous solubility of the UV absorber markedly increased. These findings are in agreement with previously published research that suggests cyclodextrins enhance transdermal permeation of topically applied drug formulations.[14,17,30]

For the 10% HPCD formulation, all drug was in solution and the concentration of oxybenzone in the solid state was zero. Flux was shown to be significantly higher than that of the 5% HPCD formulation, and these findings were again attributed to the increase in aqueous solubility of oxybenzone. The solubility of the UV absorber in the 10% HPCD formulation increased twofold compared to the 5% HPCD formulation. Flux is proportional to the concentration of drug applied to the skin and, for suspensions, the applied concentration is the maximum solubility of the drug in the vehicle. Therefore, the applied oxybenzone concentration for the 10% HPCD solution was greater than that for the 5% HPCD suspension.

Ultraviolet absorbers such as oxybenzone function by filtering and absorbing solar radiation. These chemicals must remain on the surface or in the outermost layers of the skin to be effective.[4] In the current study, the rate of oxybenzone penetration into the skin was greater than the rate of permeation through the skin, thus an increase in skin accumulation was observed for the 5 and 10% HPCD formulations. These results were presumably due to the hydrated state of the drug when complexed with HPCD. No experiments, however, were performed to determine the location of drug in the skin. Since the stratum corneum is the primary bar-

rier to skin penetration, an assumption that oxybenzone accumulated in this region of the epidermis can be made. Relatively low concentrations of HPCD, therefore, have a potential to improve photoprotection of currently existing sunscreen products, and additional in vivo UV exposure experiments are necessary. This method to increase skin accumulation of other UV absorbers or topically applied drugs, however, may not be widely applicable, since the rate of skin penetration is dependent on a number of physicochemical characteristics of the drug.[1]

Interestingly, when the HPCD concentration was increased to 20%, both the flux and skin accumulation of oxybenzone significantly decreased compared to the 10% HPCD formulation. The applied concentration of oxybenzone was identical in both of these solutions. The higher HPCD concentration shifted the theoretical equilibrium of the complexation reaction towards the complexed form (see Fig. 6). Each oxybenzone molecule had a greater chance of complexing with HPCD than penetrating into the skin. These findings are in agreement with other researchers who found high concentrations of the cyclodextrins reduced transdermal permeation.[29,31] The findings from the current study indicate that a drug reservoir may be created on the skin surface at these higher HPCD concentrations and suggest a possible mechanism to achieve a sustained effect of sunscreens or other topically applied drugs. In a previous study, Scalia and coworkers[13] showed that butylmethoxydibenzoylmethane (BM-DBM), another UV absorber, complexed with HPCD and that the complex was significantly more stable to light. Since these UV absorbers function by reacting with light to protect the skin, the photoprotective effects of the complexed oxybenzone still must be addressed.

CONCLUSIONS

The current study showed that hydroxypropyl-β-cyclodextrin complexed oxybenzone, thereby significantly increasing the aqueous solubility of the drug. Based on the phase–solubility diagram, a 1:1 stoichiometry for the complexation was assumed and a stability constant of $2047\,M^{-1}$ was calculated. Flux, transdermal permeation, and skin accumulation of oxybenzone were low and found to increase with increasing concentration of HPCD up to 10%. Maximum flux occurred at 10% HPCD, when sufficient HPCD was added to the oxybenzone formulation to solubilize all drug. At HPCD concentrations higher than 10%, the flux, transdermal permeation, and skin accumulation of the drug decreased. These findings suggest that high concentrations of HPCD may create a drug reservoir on the skin surface.

REFERENCES

1. Hadgraft, J.; Pugh, W.J. The Selection and Design of Topical and Transdermal Agents: A Review. J. Invest. Derm. **1998**, *3*, 131–135.
2. Guy, R.H.; Hadgraft, J.; Bucks, D.A. Transdermal Drug Delivery and Cutaneous Metabolism. Xenobiotica **1987**, *17*, 325–343.
3. Barry, B. Penetration Enhancers: Mode of Action in Human Skin. CIRD Symposium, Advances in Skin Pharmacology–Skin Pharmacokinetics, 1986.
4. Patel, N.; Highton, P.M. Properties of Topical Sunscreen Formulations. J. Derm. Surg. Oncol. **1992**, *18*, 316–320.
5. Treffel, P.; Gabard, B. Skin Penetration and Sun Protection Factor of Ultraviolet Filters from Two Vehicles. Pharm. Res. **1996**, *13*, 770–774.
6. Hagedorn-Leweke, U.; Lippold, B.C. Absorption of Sunscreens and Other Compounds Through Human Skin In Vivo: Derivation of a Method to Predict Maximum Fluxes. Pharm. Res. **1995**, *12*, 1354–1360.
7. Okereke, C.S.; Barat, S.A.; Abdel-Rahman, M.S. Safety Evaluation of Benzophenone-3 After Dermal Administration in Rats. Tox. Lett. **1995**, *80*, 61–67.
8. Loftsson, T.; Guomundsdottir, H.; Sigurjonsdottir, J.F.; Sigurosson, H.H.; Sigfusson, S.D.; Masson, M.; Stefansson, E. Cyclodextrin Solubilization of Benzodiazepines: Formulation of Midazolam Nasal Spray. Int. J. Pharm. **2001**, *212*, 29–40.
9. Loftsson, T.; Brewster, M.E. Pharmaceutical Applications of Cyclodextrins. 1. Drug Solubilization and Stabilization. J. Pharm. Sci. **1996**, *85*, 1017–1025.
10. Nagase, Y.; Hirata, M.; Wada, K.; Arima, H.; Hirayama, F.; Irie, T.; Kikuchi, M.; Uekama, K. Improvement of Some Pharmaceutical Properties of DY-9760e by Sulfobutyl Ether β-Cyclodextrin. Int. J. Pharm. **2001**, *229*, 163–172.
11. Williams R.O.; Liu, J. Influence of Formulation Technique for Hydroxypropyl-β-Cyclodextrin on the Stability of Aspirin in HFA 134a. Eur. J. Pharm. Biopharm. **1999**, *47*, 145–152.
12. Antoniadou-Vyza, E.; Buckton, G.; Michaleas, S.G.; Loukas, Y.; Efentakis, M. The Formation of an Inclusion Complex of Methocarbamol with Hydroxypropyl-β-Cyclodextrin: The Effect on Chemical

Stability, Solubility and Dissolution Rate. Int. J. Pharm. **1997**, *158*, 233–239.
13. Scalia, S.; Villani, S.; Scatturin, A.; Vandelli, M.A.; Forni, F. Complexation of the Sunscreen Agent, Butyl-Methoxydibenzoylmethane, with Hydroxypropyl-β-Cyclodextrin. Int. J. Pharm. **1998**, *175*, 205–213.
14. Legendre, J.Y.; Rault, I.; Petit, A.; Luijten, W.; Demuynck, I.; Horvath, S.; Ginot, Y.M.; Cuine, A. Effects of β-Cyclodextrins on Skin: Implications for Transdermal Delivery of Piribedil and a Novel Cognition Enhancing-Drug, S-9977. Eur. J. Pharm. **1995**, *3*, 311–322.
15. Williams, A.C.; Shatri, S.R.S.; Barry, B.W. Transdermal Permeation Modulation by Cyclodextrins: A Mechanistic Study. Pharm. Dev. Technol. **1998**, *3*, 283–296.
16. Loftsson, T.; Sigurdardottir, A.M.; Olafsson, J.H. Improved Acitretin Delivery Through Hairless Mouse Skin by Cyclodextrin Complexation. Int. J. Pharm. **1995**, *115*, 255–258.
17. Loftsson, T.; Masson, M.; Sigurdsson, H. Cyclodextrins and Drug Permeability Through Semi-Permeable Cellophane Membranes. Int. J. Pharm. **2002**, *232*, 35–43.
18. Kastin, G.; Bowman, L. Electrical Analysis of Fresh, Excised Human Skin: Comparison with Frozen Skin. Pharm. Res. **1990**, *7*, 1141–1146.
19. Pitman, I.; Downes, L. Cattle and Sheep Skin Permeability: Comparison of Frozen and Reconstituted Skin with That of Fresh Skin. J. Pharm. Sci. **1982**, *71*, 846.
20. Becket, G.; Schep, L.J.; Tan, M.Y. Improvement of the In Vitro Dissolution of Praziquantel by Complexation with α-, β-, and γ-Cyclodextrins. Int. J. Pharm. **1999**, *179*, 65–71.
21. Higuchi, T.; Connors, K.A. In *Advances in Analytical Chemistry and Instrumentation*; Reilley, C.N., Ed.; John Wiley & Sons: New York, 1965; 117–212.
22. Loftsson, T.; Frioriksdottir, H.; Thorisdottir, S.; Stefansson, E.; Siguroardottir, A.M.; Guomundsson, O.; Sigthorsson. T. 2-Hydroxypropyl-β-Cyclodextrin in Topical Carbonic Anhydrase Inhibitor Formulations. Eur. J. Pharm. Sci. **1994**, *1*, 175–180.
23. Faucci, M.T.; Mura, P. Effect of Water-Soluble Polymers on Naproxen Complexation with Natural and Chemically Modified β-Cyclodextrins. Drug Dev. Ind. Pharm. **2001**, *27*, 909–917.
24. Dalmora, M.E.; Dalmora, S.L.; Oliveira, A.G. Inclusion Complex of Piroxicam and β-Cyclodextrin and Incorporation in Cationic Microemulsion. In Vitro Drug Release and In Vivo Topical Anti-inflammatory Effect. Int. J. Pharm. **2001**, *222*, 45–55.
25. Vitoria, M.; Bentley, B.; Renata, F.; Wilson, S.; Collett, J.H. Characterization of the Influence of Some Cyclodextrins on the Stratum Corneum from the Hairless Mouse. J. Pharm. Pharmacol. **1997**, *49*, 397–402.
26. Siefert, B.; Keipert, S. Influence of Alpha-Cyclodextrin and Hydroxyalkylated β-Cyclodextrin Derivatives on the In Vitro Corneal Uptake and Permeation of Aqueous Pilocarpine HCl Solutions. J. Pharm. Sci. **1997**, *86*, 716–720.
27. Arima, H.; Miyaji, T.; Irie, T.; Hirayama, F.; Uekama, K. Possible Enhancing Mechanism of the Cutaneous Permeation of 4-Biphenylacetic Acid by β-Cyclodextrin Derivatives in Hydrophilic Ointment. Chem. Pharm. Bull. **1996**, *44*, 582–586.
28. Tanaka, M.; Iwata, Y.; Kouzuki, Y.; Taniguchi, K.; Matsuda, H.; Arima, H.; Tsuchiya, S. Effect of 2-Hydroxypropyl-β-Cyclodextrin on Percutaneous Absorption of Methyl Paraben. J. Pharm. Pharmacol. **1995**, *47*, 897–900.
29. Loftsson, T.; Masson, M. Cyclodextrins in Topical Drug Formulations: Theory and Practice. Int. J. Pharm. **2001**, *225*, 15–30.
30. Vollmer, U.; Muller, B.W.; Mesens, J.; Wilffert, B.; Peters, T. In Vivo Skin Pharmacokinetics of Liarozole: Percutaneous Absorption Studies with Different Formulations of Cyclodextrin Derivatives in Rats. Int. J. Pharm. **1993**, *99*, 51–58.
31. Loftsson, T.; Siguroardottir, A.M. The Effect of Polyvinylpyrrolidone and Hydroxypropyl Methylcellulose on HP-β-CD Complexation of Hydrocortisone and Its Permeability Through Hairless Mouse Skin. Eur. J. Pharm. Sci. **1994**, *2*, 297–301.