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Research Paper

# Using cyclodextrin complexation to enhance secondary photoprotection of topically applied ibuprofen

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**Abstract**

Each year millions of people are overexposed to the sun resulting in photodamage of the skin. Secondary photoprotection is the application of medicinal agents to the body *after* sun exposure to reduce this damage. The objective of this study was to determine the affects of hydroxypropyl-β-cyclodextrin (HPCD) complexation on the secondary photoprotective properties of topically applied ibuprofen. Complexation of ibuprofen by HPCD was demonstrated by differential scanning calorimetry, while solubilities were determined using HPLC. A linear ($r^2 > 0.999$) relationship was found between ibuprofen solubility and HPCD concentration. For subsequent experiments, the concentration of ibuprofen was held constant at the solubility in 10% HPCD (10.6 mg/ml), while the HPCD concentration varied from 0 to 20% (w/w). In vitro transdermal permeation experiments demonstrated a parabolic relationship between transdermal kinetic parameters and HPCD concentration, with maximum values for both flux and skin accumulation occurring with the 10% HPCD formulation. In vivo experiments were performed by exposing hairless mice to UV radiation and applying ibuprofen–HPCD formulations topically at various times following UV exposure. Edema and epidermal lipid damage data demonstrated that application of ibuprofen–HPCD formulations within 1 h of UV exposure provided significant photoprotection.


*Keywords:* Cyclodextrin; Photoprotection; Ultraviolet radiation; Transdermal; Skin accumulation; Ibuprofen; Complexation

## 1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, non-selectively inhibit cyclooxygenase enzymes responsible for the conversion of arachidonic acids to prostaglandins [1]. The anti-inflammatory properties of these drugs make them suitable for the treatment of headaches, muscle aches, and arthritis [2]. In addition, recent human epidemiological studies suggest that regular oral administration of NSAIDs has preventative effects against colon, breast, and prostate cancers [3]. Cell culture and animal studies have provided additional evidence of the anti-skin cancer activity of NSAIDs [4]. Chronic oral use of these compounds, however, has been associated with a number of side effects including decreased gastric cytoprotection, impairment of renal function, and inhibition of platelet aggregation [5].

Overexposure of skin to solar radiation results in an induction of the cyclooxygenase enzyme system [6] and a subsequent inflammatory response consisting of erythema and edema that peak 12–24 h post-exposure [7]. This is commonly characterized as 'sunburn'. Another consequence of this inflammatory reaction is the production of reactive oxygen species and other free radicals thought to be important in the promotion and progression of skin cancer [8]. Despite the best efforts of organizations such as the American Cancer Society in providing sun safety education to the public, millions of people are overexposed to the sun every year. The use of sunscreens and sun avoidance behavior modifications are considered *primary prevention*. While sunscreens do provide excellent protection against solar radiation, they are ineffective if not applied prior to sun exposure and reapplied periodically during exposure. *Secondary photoprotective* agents are substances that prevent or reduce the damaging effects of solar radiation *after* sun exposure. Various classes of compounds (i.e.

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antioxidants, isoflavones, and anti-inflammatory drugs) have demonstrated photoprotectant properties by acting at various points in the oxidative damage and inflammatory response pathways that are crucial in the development of skin cancer [9]. Regardless of the therapeutic class of compound, all must reside *within* the skin to be effective. Once the compounds have permeated to the systemic circulation, the therapeutic effects of the drug diminish rapidly. Topical non-steroidal ant-inflammatory drug (NSAID) therapy could have potential secondary preventative effects against skin damage caused by the sun via the localized inhibition of cyclooxygenase and subsequent decreased inflammatory response if accumulation in the skin could be maximized.

One method that our laboratory has been investigating to alter the transdermal kinetics of topically applied drugs is cyclodextrin complexation [10]. Cyclodextrins are crystalline, cyclic oligosaccharides with a bucket-like structure having a hydrophobic internal cavity and a hydrophilic exterior cavity that allows the formation of inclusion complexes, in which lipophilic compounds are non-covalently bound within the cavity. Cyclodextrins have been employed in the pharmaceutical industry to increase the aqueous solubility and stability of drugs and have been used in both parenteral and oral drug delivery systems [11]. The objective of this study was to use cyclodextrin complexation to alter the transdermal kinetics of a model NSAID and determine the drug–HPCD complex's potential for secondary photoprotection.

## 2. Materials and methods

### 2.1. Materials

Ibuprofen was purchased from Spectrum Chemical Company (Gardena, CA). 2-Hydroxypropyl-β-cyclodextrin (0.8 molar substitution, HPCD), monobasic sodium phosphate, aqueous formaldehyde solution (37%), and Brij® 58 (polyoxyethylene [20] acetyl ether) were purchased from Sigma-Aldrich (Milwaukee, WI). Acetonitrile, methanol, and glacial acetic acid were purchased from Fisher Scientific Company (Fair Lawn, NJ). Chemicals were used as received.

### 2.2. Determination of ibuprofen aqueous solubility

The maximum aqueous solubility of ibuprofen was determined in 0, 5, 10, and 20% (w/w) solutions of HPCD. An excess of ibuprofen was added to deionized water–HPCD solutions. The resulting suspensions were covered with Parafilm® and magnetically stirred for 24, 48, 96 or 168 h at room temperature. The mixtures were then centrifuged at 3300 rpm (142 g) for 10 min and an aliquot of the supernatant was analyzed for ibuprofen content using high-performance liquid chromatography (HPLC).

### 2.3. Determination of complexation

A modulated differential scanning calorimeter (Model 2920, TA Instruments, New Castle, DE) was used to demonstrate ibuprofen–HPCD complexation. Approximately 10 mg of ibuprofen was sealed in aluminum pans and scanned at a rate of 10 °C/min from 20 to 100 °C to determine melting point. The modulation signal was set at 1.592 °C/min. The thermogram was analyzed using TA Instruments Universal Analysis software. To confirm complexation of ibuprofen, aqueous solutions containing the maximum amount of ibuprofen soluble at 10% HPCD were prepared as described in Section 2.2 and the solvent was evaporated by placing the solution in a vacuum oven at 50 °C overnight. The resulting powder was analyzed for the presence or absence of a melting point, with the absence of a melting transition indicative of complexation. An additional experiment was performed by analyzing the drug after being dissolved and the solvent evaporated.

### 2.4. Preparation of ibuprofen–HPCD topical formulations

Simple aqueous solutions and suspensions were prepared for transdermal permeation and skin accumulation studies by mixing ibuprofen for 24 h in 0, 5, 10, or 20% (w/w) solutions of HPCD. The concentrations of ibuprofen were held constant at 10.6 mg/ml for all formulations, which were equal to the maximum aqueous solubility of ibuprofen in a 10% HPCD solution. Thus, formulations containing 0 or 5% HPCD were suspensions, while solutions were formed at 10 and 20% HPCD.

### 2.5. In vitro transdermal permeation

Hairless mouse skin was used as the model for the in vitro transdermal permeability and skin accumulation studies. All animal experiments described were reviewed and approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee. Six-week-old male SKH-1 hairless mice were obtained from Charles Rivers Laboratories (Wilmington, MA). Animals were sacrificed by $CO_2$ asphyxiation and full-thickness abdominal and dorsal skin was excised. After extraneous subcutaneous fat was removed from the dermal surface; the animal skins were placed in sealable freezer bags and stored at −10 °C (Revco Scientific, Asheville, NC). 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 [12,13]. Immediately prior to experimentation, the skin was thawed at 37 °C for approximately 15 min. Skin samples were then mounted on 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 ± 0.5 °C and continuously stirred at 600 rpm using magnetic stirrers. Following a 1-h hydration period, 200 μl of an ibuprofen–HPCD formulation was applied to each skin. Samples of the receptor phase (300 μl) were withdrawn at specified time points over a 24-h period and stored at −10 °C until analyzed using HPLC. The receptor fluid withdrawn was immediately replaced with fresh buffer and analysis of samples was corrected for previous drug removed.

### 2.6. In vitro skin accumulation

Skin accumulation of ibuprofen was determined at 2, 4, 12, and 24 h following application of the drug formulations. Residual formulation on the surface of the skin was removed using a cotton tip applicator. The donor cap was then disconnected from the Franz cell and each skin sample was removed, briefly rinsed in methanol and patted dry with a lint-free wipe. The skin samples were then weighed, minced using scissors, 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 (142 g) 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.

### 2.7. HPLC chromatographic method

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 ultraviolet light absorbance detector (Model UV 1000, Thermoseparations Products). The system was controlled and the data integrated by a personal computer using chromatography management software (PC 1000, Thermoseparations Products). The chromatographic column was of 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 detection wavelength was 254 nm. The mobile phase was 60% acetonitrile and 40% monobasic sodium phosphate buffer (pH 3) at a flow rate of 1 ml/min. Retention time for ibuprofen was approximately 3.8 min and the limit of detection was 500 ng/ml. Intra and interday coefficients of variation were 2.2 and 8.7%, respectively.

### 2.8. In vivo ultraviolet radiation exposure and ibuprofen treatment

The hairless mouse model was used for the secondary photoprotection studies. All animal experiments described were reviewed and approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee. For each experimental group ($n = 4$), unrestrained mice were placed in a clear plastic box, covered with a barred lid and irradiated. UV radiation was produced by an Oriel 1000 Watt Solar Ultraviolet Simulator (Oriel Corp., Stratford, CT). This solar simulator produces a high-intensity UV beam that provides simulated sunlight in the UVA (320–400 nm) and UVB (280–320 nm) regions. Irradiance from this simulator is 112 W/m$^2$ in the UVA range and 5.18 W/m$^2$ in the UVB range with atmospheric attenuation filters employed. This energy correlates to a maximum ultraviolet (UV) radiation exposure rate of approximately 90 MED/h for hairless mouse skin at maximum energy output. The minimal erythemal dose (MED) of UV radiation for the hairless mouse is approximately 140 mJ/cm$^2$ [14] and mice were exposed to 140 or 280 mJ/cm$^2$ (equivalent to 1.0 or 2.0 MED, respectively). One MED is defined as the amount of UV radiation necessary to cause a slight reddening of the skin 24 h after exposure. At 0, 0.5, 1, or 2 h post-UV exposure, the HPCD–ibuprofen formulations were applied at 2 mg/cm$^2$. Control mice were left untreated following UV radiation exposure.

### 2.9. Quantification of in vivo edema

Immediately after UV exposure (described above), each mouse was anesthetized with ketamine (100 mg/kg IP) and skin fold thickness measurements were taken with a spring loaded pocket thickness gauge (no. 7309, Mitutoyo Corporation, Kawasaki, Kanagawa, Japan). Three measurements were taken for each mouse and these data were used as the baseline. Twenty-four hours after UV exposure, skin fold thickness measurements were taken immediately after the animals were sacrificed by $CO_2$ asphyxiation. Edema was calculated as the difference in skin fold thickness between the baseline and 24-h post-UV exposure data.

### 2.10. Quantification of in vivo epidermal lipid damage

Immediately after the second skin fold thickness measurements, full-thickness dorsal skin from each mouse was removed by blunt dissection. The epidermis was separated from the full-thickness skin by placing the skin dermis side down on filter paper saturated with a 2.5% (w/v) trypsin solution. After storage at 37 °C for 4 h, epidermal sheets were gently lifted from the skin using forceps and then covered with fresh trypsin solution and stored at 37 °C for 1 h. Trypsin was removed from the epidermal sheets with gentle rinsing using deionized water. The epidermal samples were then stored at room temperature and 75% relative humidity overnight. Following storage, approximately 10 mg samples of epidermis were sealed in aluminum pans and analyzed using differential scanning calorimetry (Model 2920 Modulated Differential Scanning Calorimeter, TA Instruments). The samples were scanned at a heating rate of 5 °C/min from 10 to 80 °C with

a temperature modulation of 0.759 °C/min. Lipid melting temperatures were determined using TA Instruments Universal Analysis software, with the endothermic peak quantified using peak integration and a linear baseline.

### 2.11. Data analysis

Amount of drug (μg) in the Franz cell receptor compartment (corrected for sample removal) was plotted against time (h). Transdermal flux was calculated by multiplying the slope of the permeation–time graph by the concentration of drug applied to the skin and is reported as microgram/square centimeter per hour. Skin accumulation was calculated by dividing the amount of drug remaining in the skin by the weight of the skin sample and is reported as microgram of drug/milligram of skin. The mean and standard deviation ($n=4$) of these parameters were calculated. For UV photoprotection studies, the means and standard deviations of lipid melting temperature and edema were determined. Statistical analysis was carried out using SigmaStat 2.0 software (SPSS, Inc., Chicago, IL). For statistical comparison, a one-way analysis of variance test was employed and a pairwise multiple comparison (Tukey) post-test was then used to determine differences between treatment groups. A $P<0.05$ was considered statistically significant.

## 3. Results

### 3.1. Determination of cyclodextrin complexation

In the current study, modulated differential scanning calorimetry was used to demonstrate HPCD complexation of ibuprofen. Thermograms revealed that ibuprofen powder and ibuprofen that had been dissolved and recrystallized both had melting points of approximately 81 °C (Fig. 1A and B). Powder obtained from solvent evaporation of an aqueous solution of HPCD and ibuprofen displayed no melting transition (Fig. 1C). The absence of melting transition in the ibuprofen–HPCD mixtures was indicative of cyclodextrin complexation. These results are in agreement with previous research that demonstrated cyclodextrin complexation of ibuprofen using other analytical techniques including phase solubility analysis, nuclear magnetic resonance, X-ray powder diffractometry, and infrared spectroscopy [15,16].

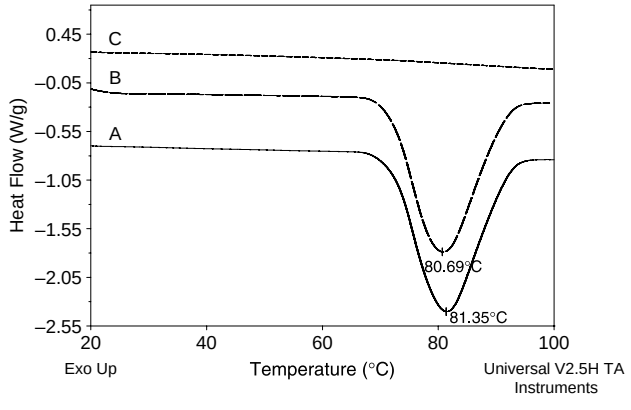


Fig. 1. Representative thermograms of (A) ibuprofen powder, (B) powder from solvent evaporation of an aqueous solution of ibuprofen, and (C) powder from solvent evaporation of an aqueous solution of ibuprofen and HPCD.

### 3.2. Influence of complexation on aqueous solubility of ibuprofen

All solubilities were reported after 24 h of stirring. No significant increases in the solubilities of ibuprofen were seen at equilibration times up to 168 h (7 days), indicating that 24 h was sufficient time for the drug to fully complex with HPCD. Ibuprofen was very sparingly soluble in water (0% HPCD), with a concentration of 0.064 mM. Increasing concentrations of HPCD significantly increased the aqueous solubility of ibuprofen in a linear fashion ($r^2>0.998$) to 99.4 mM at 20% HPCD (Fig. 2). Thus, the solubility of ibuprofen increased 1553-fold and these results are in agreement with previous studies, which found linear relationships between drug solubility and HPCD concentration [10,17].

### 3.3. Influence of complexation on transdermal permeation

The influence of complexation on the transdermal flux of ibuprofen through skin is presented in Fig. 3. The relationship between ibuprofen flux and HPCD concentration was found to be parabolic and the data are in agreement with previous studies using cyclodextrins to complex other compounds [10,18,19]. The flux values of ibuprofen from 0 ($2.7\pm0.3$ μg/cm$^2$/h) and 20% ($2.4\pm0.4$ μg/cm$^2$/h) HPCD formulations were both low and not significantly different ($P>0.05$) from each other. Maximum flux of ibuprofen occurred with the 10% HPCD formulation ($17.9\pm1.4$ μg/cm$^2$/h), but was not statistically different from the 5% HPCD formula ($15.4\pm1.4$ μg/cm$^2$/h).

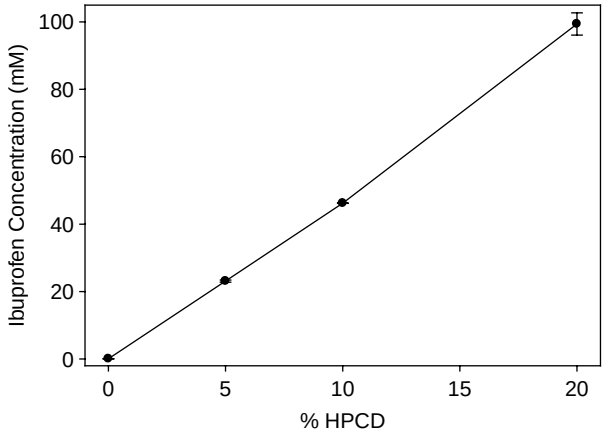


Fig. 2. The effect of HPCD concentration on the aqueous solubility of ibuprofen.

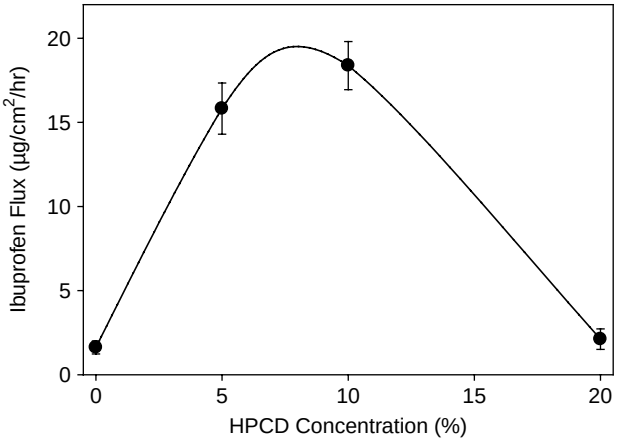


Fig. 3. Influence of HPCD concentration on the transdermal flux of ibuprofen.

### 3.4. Influence of complexation on skin accumulation

Skin accumulation data of ibuprofen over a 24-h period are presented in Fig. 4. Accumulation demonstrated a parabolic pattern similar to flux. Very little ibuprofen accumulated in the skin with 0, 5, and 20% HPCD formulations and no significant differences were found at any of the time points investigated (2, 4, 12, or 24 h). Significantly higher skin accumulations were found with the 10% HPCD formulation, with the highest values resulting from the 10% formulation at 12 ($5.3 \pm 1.1$ μg/mg) and 24 h ($5.4 \pm 0.9$ μg/mg) post-application.

### 3.5. Influence of skin accumulation of ibuprofen on secondary prevention of edema

UV exposure-induced 24-h edema data are presented in Fig. 5. Control (no ibuprofen treatment) animals exhibited significant swelling at both 1 and 2 MED UV exposures, with increases in skin fold thickness to $43.3 \pm 4.7$ and $53.3 \pm 3.7$ μm, respectively. Application of HPCD–ibuprofen

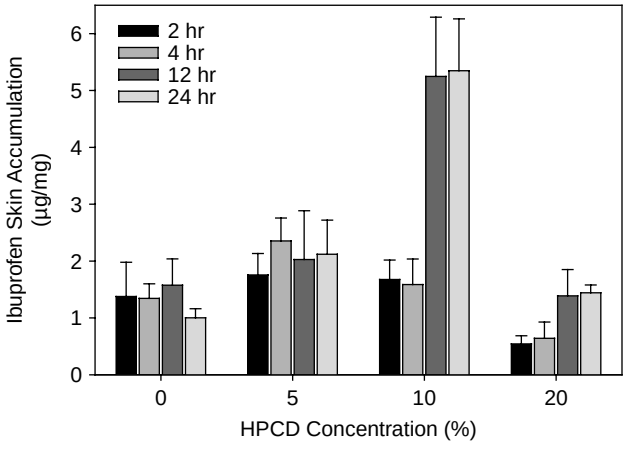


Fig. 4. Influence of HPCD concentration on skin accumulation of ibuprofen at 2, 4, 12, and 24 h.

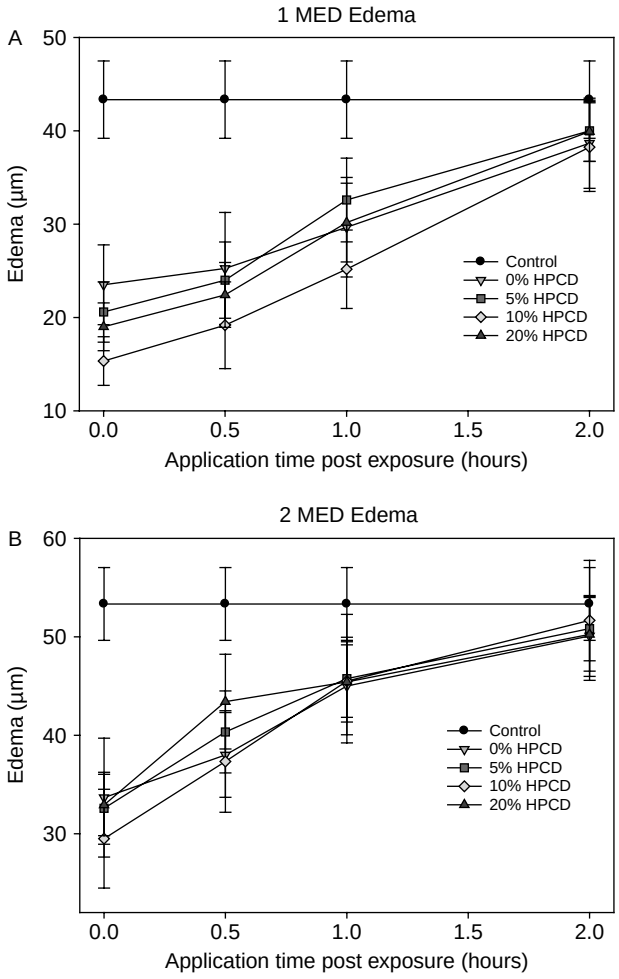


Fig. 5. Effects of HPCD concentration and application time after exposure to UV radiation-induced skin inflammation.

formulations as secondary photoprotection following a mild UV exposure of one MED revealed a direct relationship between post-exposure application time and the degree of photoprotection (Fig. 5A). Application of any of the ibuprofen–HPCD formulations at 0, 0.5 or 1 h post-UV exposure resulted in significant decreases in edema when compared to control, while application at 2 h post-UV exposure did not produce significant decreases in swelling. Comparisons between the topical formulations demonstrated that the 10% HPCD–ibuprofen formulation produced the greatest protection against edema, with these differences being significant over all other formulations. Importantly, comparisons between consecutive time points of post-exposure revealed significant differences between 0 and 0.5 h, as well as 0.5 and 1 h, indicating that time lag of ibuprofen treatment post-UV exposure is critical in providing secondary photoprotection.

Similar results were found in secondary photoprotection following a moderate UV exposure of two MED, although increased variability in response to ibuprofen treatment resulted in reduced significance between formulations (Fig. 5B). Although not as linear as with the one MED

UV exposures, a direct relationship was found between the degree of photoprotection and time of application of post-exposure. When application was immediately after UV exposure (0 h), all formulations produced significant photoprotection compared to control, with the 10% HPCD concentrations providing the greatest protection, although it was not significantly higher than the other formulations. Similar results were found at 0.5 and 1 h application times post-UV exposure, with all formulations producing significant reductions in swelling compared to control; however, no significant differences were found between any of the individual formulations. Finally, again similar to one MED exposure, no significant differences were found between control and any of the ibuprofen–HPCD formulations applied 2 h after UV exposure.

### *3.6. Influence of skin accumulation of ibuprofen on secondary prevention of in vivo epidermal lipid damage*

In quantifying epidermal lipid damage (presented in Fig. 6), lower melting temperatures of epidermal lipids indicated photodamage while higher temperatures demonstrate photoprotection. The epidermal melting temperature from animals not exposed to UV radiation was $75.7 \pm 1.0$ °C, while the epidermis from untreated (control) animals exposed to one MED of UV radiation exhibited a melting temperature of $48.6 \pm 3.4$ °C. This decrease in temperature indicated significant UV-induced damage to the epidermal lipids. At all instances, animals treated topically with ibuprofen post-UV exposure had lipid melting temperatures significantly lower than unexposed animals, indicating that photodamage had occurred despite treatment with ibuprofen (Fig. 6A). However, ibuprofen treatment at 0, 0.5 and 1 h post-exposure did result in some degree of secondary photoprotection, as indicated by epidermal lipid melting temperatures that were significantly higher than skin from untreated animals exposed to UV radiation. No significant differences in melting temperature were found between control and animals treated with ibuprofen 2 h post-exposure. Comparisons between formulations revealed that the 10 and 20% HPCD formulations showed the largest degree of secondary photoprotection when applied 0, 0.5, and 1 h post-exposure, although these values were not statistically significant compared to the other formulations. Interestingly, no significant differences were found when comparing consecutive time points of post-exposure, indicating that time of application did not play a key role in determining the degree of secondary photoprotection provided by the HPCD–ibuprofen formulations.

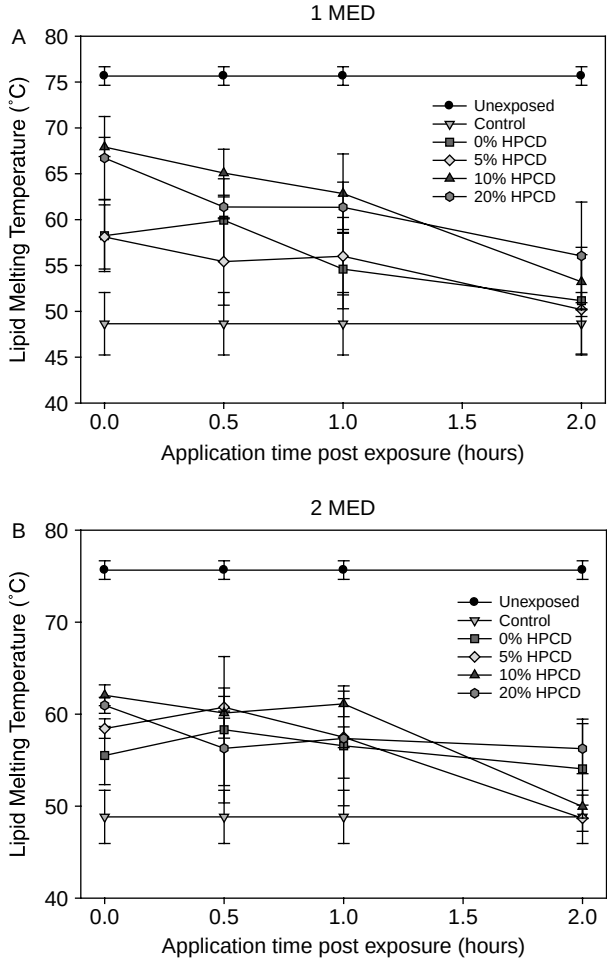


Fig. 6. Effects of HPCD concentration and application time after exposure to UV radiation epidermal lipid damage.

Photoprotection provided by the HPCD–ibuprofen formulations for the moderate two MED UV exposure (Fig. 6B) was not as pronounced when compared to the one MED exposures. While data were similar to the one MED exposures in that all formulations provided some degree of photoprotection compared to control, no significant differences were seen between formulations at any time point, illustrating that skin accumulation of ibuprofen did not influence the degree of epidermal damage. In addition, no significant differences were found between consecutive time points for any of the formulations, suggesting that time of application was also not a factor in achieving secondary photoprotection.

## 4. Discussion

Lipophilic compounds bind non-covalently to the hydrophobic interior cavity of the cyclodextrin molecule to form inclusion complexes. These complexes alter various properties of drugs including aqueous solubility and chemical stability [20,11]. Conflicting reports exist in the literature as to the relationship between cyclodextrin complexation and transdermal kinetics of topically applied compounds. Several researchers have shown cyclodextrins functioned as penetration enhancers and increased the flux of drugs across the skin [17,21], while others reported no such increases [22]. However, the majority of published studies employed drug–cyclodextrin mixtures with the drug

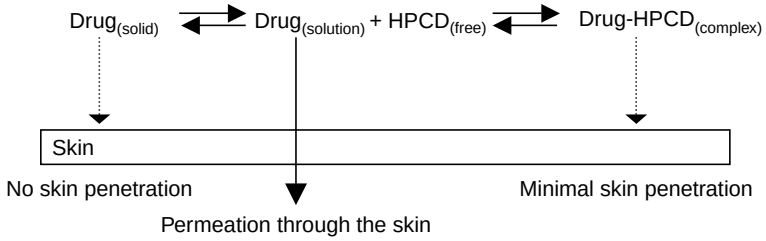


Fig. 7. Schematic representation of the complexation and transdermal permeation of drug–HPCD systems.

at maximum solubility. The current study investigated the effects of varying concentrations of HPCD on transdermal kinetics, while maintaining constant levels of ibuprofen in the formulations.

A theoretical model of the complexation and transdermal permeation of topically applied compounds complexed with HPCD (Fig. 7) can be used to predict their disposition into and through the skin. In a suspension for a given HPCD concentration, equilibria are established between solid and drug in solution and between free and complexed drug. Only free drug in solution is capable of penetration into and through the skin. In the current experiments, the concentration of ibuprofen was maintained at its maximum solubility in a 10% HPCD formulation (10.6 mg/ml). Lower concentrations of HPCD (0 and 5%) shifted the equilibrium towards the solid drug, while higher concentrations (20%) shift the equilibrium towards the complexed form. Cyclodextrins are thought to enhance drug permeation by increasing solubility and acting as a carrier of drug from aqueous solutions towards the lipophilic surface of the skin, where the drug partitions from the complex into the skin [23]. However, the addition of cyclodextrin in excess of that needed to solubilize the drug results in a decrease in the thermodynamic driving force needed for skin permeation since the equilibrium is shifted towards the complexed form [24]. Data from the current study showed that increasing the cyclodextrin concentration from 5 to 10% increased ibuprofen flux through the skin 16.5% and increased skin accumulation at 24 h by 152%. Increasing the HPCD concentration further to 20% reduced the flux by 88% and 24-h skin accumulation was reduced by 73% in comparison to the 10% HPCD formulation.

The alterations in transdermal kinetics were shown to directly influence the degree of secondary photoprotection provided by the ibuprofen–HPCD formulations, especially at lower UV radiation exposures. The inflammatory reaction in the skin caused by UV exposure is a cascade of archadonic acid and prostaglandin production resulting in the common symptoms of sunburn (edema and erythema) peaking from 12 to 24 h post-exposure [7]. UV radiation exposure to the skin results in cutaneous inflammation accompanied by the infiltration of neutrophils and a vascular injury. Keratinocytes release a variety of cytokines including tumor necrosis factor, interleukin-1 and interferon-$\gamma$. This cytokine-induced inflammatory response produces changes in vascular permeability and the activation of immune cells in the blood (neutrophils, monocytes, macrophages, and lymphocytes) and tissue (mast cells) [25]. Early interruption of these cascades and cellular activation could potentially reduce UV radiation-induced inflammation and edema.

An association was found between rapid skin accumulation of ibuprofen and the degree of secondary photoprotection. Fig. 4 shows no significant differences between skin accumulation at 2 and 4 h after application regardless of the formulation applied and, similarly, Figs. 5 and 6 show no significant differences in secondary photoprotection between formulations when applied within 1 h of UV exposure. This effect was particularly evident with mild (one MED) UV exposures. Exposure to higher, moderate levels of UV radiation (two MED) produced such a substantial inflammation that the topically applied ibuprofen was unable to prevent subsequent edema. At both UV exposures, the 10% HPCD formulation provided the highest level of photoprotection at 0, 0.5 and 1-h post-exposure application. These data would suggest that while initial penetration and accumulation of ibuprofen in the skin is important in providing secondary photoprotection, higher skin accumulation at later time points (i.e. 12 and 24 h) seen with the 10% HPCD formulation may provide enhanced protection by reducing the inflammatory reaction for extended periods of time. Data from these experiments also revealed that a lag time of 2 h post-UV exposure to apply ibuprofen to the skin provides no significant secondary photoprotection against UV radiation-induced edema, presumably due to the cascade of inflammatory events progressing to the point where application of an NSAID could not halt the further production of inflammatory compounds.

There is considerable evidence suggesting that UV radiation-induced generation of reactive oxygen species (ROS), such as singlet oxygen, hydroxyl radical and superoxide radical, results in damage to various components of the skin [26]. One significant consequence of ROS production in skin is lipid peroxidation that comprises the oxidative degradation of glycerophosphlipids, sphingolipids, unsaturated free fatty acids and cholesterol [27]. The skin is an especially susceptible target organ to UV radiation-induced lipid peroxidation as it is the outer covering of the body and is exposed almost daily to damaging UV radiation from the sun. In addition, the skin is rich in the very lipids that are targets of lipid peroxidation [28]. Finally, UV radiation has been shown to lower

the level of endogenous antioxidant enzymes present in the skin including glutathione reductase and α-tocopherol [29]. In addition to ROS produced directly from UV exposure of skin components, immune cells activated by UV exposure can also produce ROS, which can further amplify the inflammatory reaction as well as cause damage to other cellular components of the skin [30]. In short order, a potentiating loop of UV radiation exposure, inflammatory reaction and resulting oxidative stress are generated [31]. In addition, studies have suggested that lipid peroxidation can also alter the physicochemical structure of lipids allowing increased fluidity [29,32]. Thermoanalytical studies using differential scanning calorimetry have correlated these increases in lipid fluidity to decreases in epidermal melting temperatures [33,34]

In the current study, both epidermal lipid melting temperature and edema exhibited similar secondary photoprotection patterns. Significant secondary photoprotection compared to untreated animals was provided when ibuprofen was applied within 1 h of UV exposure for all formulations, while a delay of 2 h post-UV exposure eliminated this protection. Photoprotection was also more pronounced after mild (one MED) UV exposure in comparison to the moderate (two MED) exposure. Again similar to edema data, the 10% HPCD formulation provided the highest level of secondary photoprotection when applied 0, 0.5, and 1-h post-UV exposure, although these differences were not significant. These data again suggest that application of ibuprofen immediately following UV exposure is crucial in reducing epidermal lipid damage.

In general, ibuprofen provided greater photoprotection against UV radiation-induced edema than epidermal lipid damage. As discussed previously, edema is a direct result of the activation of the immune system and a cascading production of cytokines, prostaglandins, and arachidonic acid. As a NSAID, ibuprofen can interrupt these cascading events and reduce the subsequent swelling of the skin. Therefore, the earlier the drug is applied to the skin post-UV exposure, the earlier this cascade can be halted. In contrast, the production of ROS from UV exposure of skin is a two-pronged process, with the reactive molecules being produced directly from UV exposure of skin components (mainly lipids) and production from the activated immune system. While the application of ibuprofen is likely able to diminish the immune system production of ROS, the direct production of ROS is unaffected. This can be seen in the lipid melting temperature data, where some photodamage was observed at all application times of post-exposure, yet the application of ibuprofen did provide some level of secondary photoprotection compared to untreated animals.

In conclusion, the current study showed that hydroxypropyl-β-cyclodextrin (HPCD) complexed with ibuprofen and significantly increased its aqueous solubility in a linear manner. Both flux and skin accumulations of ibuprofen were found to exhibit parabolic relationships with HPCD concentrations, with maximum values occurring in the 10% HPCD formulations. Data also demonstrated that the greatest degree of photoprotection was provided by the ibuprofen–HPCD formulation that delivered the greatest amount of ibuprofen to the skin (10% HPCD formulation). In vivo, UV exposure studies further revealed that secondary photoprotection provided by topically applied ibuprofen was more dependent on the time elapsed between UV exposure and application than formulation.

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