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Effects of Chemical Enhancers on Human Epidermal Membrane: Structure-Enhancement Relationship based on Maximum Enhancement ($E_{\max}$)

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Abstract

Chemical penetration enhancers are widely used in transdermal pharmaceuticals as well as cosmetic products. Selection of suitable enhancers in topical formulations requires an understanding of the mechanism of action of these enhancers. The objective of the present study was to evaluate the enhancement effects of a number of commonly known enhancers and cosmetic ingredients on permeation across human epidermal membrane (HEM). The potencies of these chemical enhancers—maximum enhancement, $E_{\max}$ —were compared at their highest thermodynamic activity in equilibrium with HEM (i.e., solubility equilibrium). This was achieved by the treatment of HEM with the enhancer or phosphate buffered saline (PBS) saturated with the enhancer. Passive transport experiments were then conducted with a model permeant corticosterone to determine the effects of these enhancers on the lipoidal pathway of HEM. The results suggest that $E_{\max}$ of an enhancer is related to its octanol/water partition coefficient and its solubility in the HEM lipid domain. A relationship between enhancer $E_{\max}$ and its solubility in silicone elastomer was also observed, suggesting the use of silicone solubility to predict enhancer potency. Based on the $E_{\max}$ results, some common topical ingredients were found to be more potent enhancers than a number of well-known chemical enhancers.

Keywords

chemical enhancers; human epidermal membrane; transdermal permeation; maximum enhancement factor ($E_{\max}$)

INTRODUCTION

The effectiveness of a transdermal drug delivery system depends on its ability to deliver the drug at sufficient quantities for a therapeutic effect. In the past three decades, permeation enhancers for transdermal delivery have been extensively studied.^{1–4} Over 360 molecules have been shown to enhance the permeation of chemicals across the stratum corneum (SC) and are classified as chemical penetration enhancers. Fatty acids, terpenes, pyrrolidones, and azone are examples of commonly studied transdermal enhancers.^{5–10} In general, the mechanisms of chemical enhancers are suggested to be: (a) enhancer perturbation or fluidization of the lipid structure,¹¹ lipid extraction and solubilization,^{12,13} and the modification of the structure of the lipids in SC, (b) protein denaturing effects (such as phenol) on the desmosome thus stripping

the squames, which is a dramatic approach not widely accepted,¹⁴ and (c) denaturation of the keratin, keratinocyte swelling, and vacuolation resulting in alteration of the corneocyte in SC.

Earlier transdermal studies of chemical permeation enhancers were mainly focused on enhancer screening. Later studies are focused on understanding the mechanisms of the enhancers and synergistic effects.^{15–17} Attempts have also been made to establish a general relationship between the enhancer structures and enhancer effectiveness.^{18–20} It is generally believed that a clear understanding of the mechanism of transdermal permeation enhancement and the establishment of a structure-enhancement relationship would allow the proper selection of enhancers in transdermal formulations. The following are the important findings in our previous studies on the mechanism of transdermal chemical enhancers and their structure-enhancement relationship: (a) the potencies of the enhancers based on their concentrations in aqueous solutions are related to the enhancer octanol–water partition coefficients, (b) the enhancers and their polar head groups are distributed in the SC lipid domain with properties similar to short chain *n*-alkanols, and (c) the intrinsic potencies of the enhancers are essentially the same based on their concentrations in the SC lipid domain independent of the enhancer chemical structures.^{21–24} The present study was a continuing effort to evaluate the structure-enhancement relationship based on these findings and hypotheses.^{25,26}

The vast majority of chemical enhancers present in transdermal products are highly lipophilic. Due to the lipophilic nature of the enhancers, co-solvents were used in many enhancer studies. Although some co-solvents were shown not to affect the integrity of SC and transport experiments with the co-solvents were used as the control, it is difficult to rule out any potential synergistic effects between the enhancers and co-solvents. The study of enhancers using aqueous media is one approach to avoid possible synergy effects from co-solvents to examine the sole effects of the enhancers, but this method has its limitations. For example, the use of aqueous media to study lipophilic enhancers can be difficult due to rapid depletion of the enhancers in the aqueous media as well as the inability to determine the aqueous free enhancer concentrations. Previous studies have used solubilizing agents as reservoirs to maintain suitable concentration of lipophilic enhancers in aqueous solution.^{27,28} However, the use of solubilizing agents can affect the permeability or the integrity of SC. Even if the solubilizing agents do not affect SC permeability, a wide range of control studies is required for the mechanistic interpretation of the data.

In addition to a better mechanistic understanding of the intrinsic effects of the chemical enhancers, the strategy of not using co-solvents and solubilizing agents in an enhancer study can be useful to assess enhancers in a transdermal solution, gel, or aerosol formulation. An aerosol transdermal system has recently been studied.^{29,30} The aerosol delivery system is consisted of a fast drying topical solvent with a lipophilic enhancer, so the solvent is not expected to affect skin permeability or provide a solvent-enhancer synergistic effect after solvent evaporation. The use of co-solvents and solubilizing agents is therefore not preferred in the study of the effects of enhancers in this system.

The objectives of the present study were to determine: (a) the effectiveness of the enhancers based on the enhancer maximum enhancement effects, (b) the relationship between the potencies and lipophilicities of these enhancers, and (c) the feasibility of using enhancer solubility in silicone to predict the maximum potencies of the enhancers. The chemical permeation enhancers studied were those commonly used in transdermal products as well as cosmetic products. Permeation enhancers that had been previously studied were also included for comparison because of the unique experimental approach used in the present study. A list of the studied enhancers is provided in Table 1, and their structures are shown in Figure 1. The potencies of the enhancers were evaluated by their maximum intrinsic enhancement effects when the enhancers were presented at their highest thermodynamic activity in equilibrium with

HEM. Corticosterone was the model drug because the SC lipid domain was the predominant SC transport rate-determining pathway for corticosterone, and this allowed the direct evaluation of the effects of the chemical enhancers on the SC lipoidal pathway.

EXPERIMENTAL METHODS

Materials

³H-corticosterone (CS) at purity >97% was purchased from Perkin Elmer Life and Analytical Sciences (Boston, MA) and American Radiolabeled Chemicals, Inc. (St. Louis, MO). Isopropyl myristate and *n*-hexanol were obtained from Alfa Aesar (Ward Hill, MA) at purity >98%. Oleyl alcohol was obtained from Alfa Aesar at purity >85%. *n*-Octanol, 2-phenoxyethanol, butylated hydroxyanisole, salicylaldehyde, 1-undecanol, and sodium azide (NaN₃) were obtained at purities ≥98% from Acrôs Organics (Morris Plains, NJ). 1-Octyl-2-pyrrolidinone, N-dodecylpyrrolidinone, 2-ethyl hexylsalicylate, benzyl alcohol, and iso-menthone (menthone) at purities >98% and corticosterone at purity >92% were purchased from Sigma-Aldrich, Co. (St. Louis, MO). Padimate O was obtained at purity >90% from Spectrum Chemicals (Gardena, CA). Oleic acid was obtained from Fisher Chemicals (Pittsburgh, PA) at purity >95%. Laurocapram (azone) was purchased at 91% purity from NETQEM (Durham, NC). 1-Hexyl-2-pyrrolidinone was purchased from ISP chemical products (Milford, CT). Phosphate buffered saline (PBS: 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride) pH 7.4, was prepared by dissolving phosphate buffer tablets in distilled deionized water. PBS was preserved using 0.02% NaN₃. Silicone elastomer components (MED-6033) were purchased from NuSil Technology (Carpinteria, CA). The diffusion cells used in the transport experiments were consisted of two half cells. Each cell had a volume capacity of approximately 2 mL and diffusional area of approximately 0.8 cm².

Split thickness cadaver skin obtained from the New York Firefighters Skin Bank (New York, NY) was of the posterior torso of thirteen male donors. Human epidermal membrane (HEM), consisting of the SC and the viable epidermis, was prepared by the removal of the dermis via heat separation of the skin. Briefly, the cadaver skin was immersed in PBS at 60°C for 1 min and the dermis was peeled off from HEM.³¹ HEM was then stored in a -5°C freezer for later use. At least three skin donors were used for each enhancer in the experiments.

Theory and $E_{\max}$

The total permeability coefficient across HEM can be modeled by:

$$P_T = \frac{1}{\frac{1}{P_p + P_L} + \frac{1}{P_{\text{epi}}}} \quad (1)$$

where P_T is the total apparent permeability coefficient, P_{epi} the permeability coefficient across the viable epidermis, P_p the pore pathway permeability coefficient (and was determined in this study by electrical resistance measurements), and P_L the permeability coefficient of the lipoidal pathway. P_p and P_L represent the parallel transport pathways across SC. For the permeation of a moderate lipophilic compound, such as CS, the lipoidal pathway is the rate determining pathway and allows the approximation:

$$P_T \approx P_L \quad (2)$$

A new definition $E_{\max}$ was introduced in the present study to describe the maximum enhancement on transdermal transport in an aqueous medium that can be achieved by an

enhancer without the interference from a co-solvent. Particularly, $E_{\max}$ was the enhancement factor of the permeability coefficient of the HEM lipoidal pathway in PBS when the enhancer thermodynamic activity in HEM approached its maximum in solubility equilibrium. In the present study, $E_{\max}$ was achieved by the treatment of HEM with neat enhancer or an enhancer saturated aqueous solution, according to the assumption that a solute (in this case the enhancer) at saturation in a solution had thermodynamic activity similar to that of its pure state.³² When equilibrium was attained between HEM and the enhancer, the thermodynamic activity of the enhancer in HEM would be the same as that of a pure enhancer solvent and that of an enhancer saturated solution. Another assumption made in this approach was that the presence of PBS would not alter the interactions between HEM and the enhancer. The maximum lipoidal pathway transport enhancement factor, $E_{\max}$, was determined by the ratio of the permeability coefficients of the permeant in the presence and absence of the enhancer with HEM samples from the same donor:

$$E_{\max} = \frac{P_{L,\text{enhancer}}}{P_{L,\text{PBS}}} \frac{S_{\text{enhancer}}}{S_{\text{PBS}}} \quad (3)$$

where $P_{L,\text{enhancer}}$ is the permeability coefficient of the lipoidal pathway when the thermodynamic activity of the enhancer in HEM approaches equilibrium with the enhancer in its pure form, $P_{L,\text{PBS}}$ the permeability coefficient of the lipoidal pathway of untreated HEM in PBS. S_{enhancer} is the solubility of CS in the enhancer-PBS solution, and S_{PBS} the solubility of CS in PBS. The ratio of the solubility is used to correct for any changes in CS thermodynamic activity in the enhancer-PBS solution from PBS.

Solubility Studies

Solubility of Enhancer in PBS—The solubility of enhancer in PBS was determined at 25°C. Ten milliliters of PBS were pipetted into a pyrex culture tube. One milliliter of the enhancer (or 1 g of solid enhancer) was then added to the 10 mL, and the mixture was shaken vigorously. The culture tube was then allowed to equilibrate at $25 \pm 1^\circ\text{C}$ in a well-shaken water-bath for 48 h. The pyrex culture tubes were then centrifuged at 3400 rpm for 30 min (Fisher Centrifuge Model 228). The saturated PBS solution was analyzed to determine the enhancer concentration using HPLC or GC as described later in the paper.

Corticosterone Solubility Analysis—Five milligrams of CS were precisely weighed and placed in a pyrex culture tube with 2 mL of PBS or enhancer-PBS solution. The tubes were left in a shaking waterbath at 37°C for 72 h. After 72 h, the tubes were centrifuged for 15 min (Fisher Centrifuge Model 228). The clear supernatant was filtered using a 0.45- μm Millipore filter (MFTM membrane, Bioscience, life Science Products). The first part of the filtrate was discarded. The filtered supernatant was then diluted and analyzed with HPLC. The solubility of CS in PBS determined using this method was found to be essentially the same as those determined previously.^{24,28}

HEM Transport Experiments: PBS/Control

The HEM transport experiments were conducted in side-by-side diffusion cells as described previously.²³ Briefly, HEM was mounted in the side-by-side diffusion cell with a Millipore filter (Durapore membrane filters, 0.22 μm pore size) placed on each side of HEM as the support³³ and the SC side facing the donor and the viable epidermis facing the receiver. The filter support reduced the physical stress on HEM due to the hydrostatic pressure in the side-by-side diffusion cells. In the enhancer study, the filters could prevent the enhancer possibly remaining on HEM surface that might form droplets from getting into the diffusion cells. Two rubber gaskets and paraffin film were used to seal the interface of the two halves of a diffusion

cell. Two milliliters of PBS were pipetted in the donor and receiver chambers. HEM was then equilibrated in the well stirred side-by-side diffusion cells for 12 h at 37°C in a circulating waterbath.

Before and after equilibration, the integrity of HEM was checked by the electrical resistance of the membrane. Electrical resistance measurement was carried out by applying a direct current to provide 0.1 V across HEM as described previously.³⁴ The electrical resistance of HEM was calculated using Ohm's law. Only HEM samples with initial electrical resistance $\geq 15 \text{ k}\Omega\text{cm}^2$ were used in the study.³⁴ After equilibration, the solutions in the donor and the receiver chambers were replaced by fresh PBS. $^3\text{H-CS}$ ($\approx 0.1 \text{ }\mu\text{Ci}$) was pipetted into the donor chamber. Passive transport was conducted in the side-by-side diffusion cells under stirring. The duration of the experiments was at least four times the lag time to ensure that steady state was attained. At predetermined time points, 2 mL samples were taken from the receiver chamber and 10 μL samples from the donor. Two milliliters of fresh PBS were pipetted into the receiver to maintain its volume. The samples were then mixed with 10 mL of scintillation cocktail (Ultima Gold, Boston, MA). A liquid scintillation counter (Beckman Coulter LS 6500 Multipurpose Scintillation Counter, Fullerton, CA) was used to analyze the samples. After the transport experiments, HEM electrical resistance was checked again. This step was essential to verify that the pore pathway of HEM had maintained its integrity throughout the transport experiment.

The permeability coefficient of the model permeant, CS, across HEM was calculated²⁴ by:

$$P = \frac{1}{AC_D} \frac{dQ}{dt} \quad (4)$$

where A is the available diffusional area of the diffusion cell, C_D the concentration of the model permeant in the donor chamber, and dQ/dt the slope of the linear region in the cumulative amount of permeant in the receiver chamber against time plot representing steady-state diffusion.

HEM Transport Experiments: Enhancer Studies

In order to maintain the thermodynamic activity of an enhancer at its maximum in HEM, three protocols were used. These protocols all allowed HEM to equilibrate with an enhancer to attain E_{max} . The protocol selected for each enhancer was based on the physicochemical property of the enhancer such as its octanol/water partition coefficient (K_{oct}) as shown in Figure 2.

Transport Across HEM in Enhancer-Saturated PBS (Protocol I)

HEM was mounted in a side-by-side diffusion cell as stated above. After equilibration, PBS was removed from the diffusion cell chambers and the solution in both chambers was replaced with fresh enhancer solution. HEM was then equilibrated in the enhancer solution under stirring and by replacing the solution in the chambers with fresh enhancer solution several times ($6 \times 20 \text{ min}$ each). The enhancer solution was PBS saturated with an enhancer at 25°C prepared according to the solubility determined in the enhancer solubility study. To ensure that equilibrium had been attained, the enhancer concentration in the equilibrating solution was checked before the transport experiment, and no significant depletion of the enhancer was found. HEM was treated with the enhancer solution from both sides (donor and receiver) to provide a symmetric distribution of the enhancer in the SC. This condition was desired in the present study to avoid complicated mechanistic interpretation of the transport data because the presence of an enhancer only on one side of the skin (e.g., the SC side) would result in a concentration gradient of the enhancer in the SC. Following equilibration of HEM with the

enhancer solution, ^3H -CS was added in the donor chamber. The transport experiment was conducted as described in “HEM Transport Experiments: PBS/Control” except that the receiver was replaced with fresh enhancer solution instead of PBS after each sampling. The electrical resistance of HEM was determined three times for each HEM in these experiments using the same protocol as stated in “HEM Transport Experiments: PBS/Control”: (a) after equilibration of HEM at 37°C to ensure the integrity of the skin before enhancer treatment, (b) after equilibration of HEM with the enhancer solution to evaluate possible enhancer-induced changes in the pore pathway in HEM, and (c) after the transport experiment to check if the HEM pore pathway had remained intact throughout the experiment.

Direct Enhancer Treatment Method Development of Protocols II and III

Evaluation of Treatment Time—Protocols II and III involved the direct treatment of HEM by the immersion of HEM in the liquid enhancers. The direct enhancer treatment provided an infinite dose of the enhancer and allowed the equilibrium of the enhancer in its pure state with HEM, so the maximum equilibrium thermodynamic activity of the enhancer could be achieved in HEM. To optimize this technique, IPM, PADO, OS, UD, and OA were selected to determine the impact of treatment time on the enhancement factor. HEM was cut into squares of 1.2 cm $\times$ 1.2 cm and placed in a Petri dish containing 10 mL of PBS for 2 h. HEM was then removed from PBS and placed in 5–10 mL of the liquid enhancer in a Petri dish at room temperature ($25 \pm 2^\circ\text{C}$) for 1, 4, 20 min or 12 h. After treatment, HEM was removed from the enhancer and patted gently with Kimwipes to remove the excess enhancer on the HEM surfaces. HEM was then rinsed in 20 mL of PBS for 10 s three times. Between each rinse, HEM was patted dry using Kimwipes. The enhancer-treated HEM was mounted in the diffusion cell and was equilibrated with 2 mL of PBS at 37°C for 2 h to allow redistribution of the enhancer throughout the SC. Five hundred microliters of the equilibrating enhancer solution were removed from both diffusion cell chambers and analyzed for the enhancer concentration. With these five enhancers, the concentrations of the enhancers were below the detection limit of the present HPLC and GC assay. After equilibration, transport experiments were conducted in PBS alone without the enhancer similar to those described in “HEM Transport Experiments: PBS/Control”. The electrical resistance of HEM was determined prior to and after the transport experiment to check the integrity of HEM and determine the impact of the enhancer on the HEM pore pathway.

Examination of HEM Dehydration—The effect of skin dehydration after prolonged contact with the enhancer in its pure form was examined. The effect of direct treatment with the enhancer saturated with PBS and that without PBS saturation was compared. In this study, 20 mL of enhancer was placed in a separating funnel together with 10 mL of PBS and shaken vigorously in order to saturate the enhancer with PBS. The two phases were allowed to separate, and PBS-saturated enhancer was pipetted out from the enhancer phase. HEM was treated with 5–10 mL of the PBS-saturated enhancer in a Petri dish for 20 min or 12 h. Transport experiments with the HEM were then carried out as described in “Evaluation of Treatment Time.”

Transport Across HEM After Direct Treatment of HEM With PBS-Saturated Enhancer (Protocols II and III)

Based on the results from the above experiments, the proper procedure in Protocols II and III would be to treat HEM with PBS-saturated enhancer for 20 min. The rationale of this is provided in the Results and Discussion Section. The experiments of direct enhancer treatment were further divided into two protocols with different post-enhancer treatment equilibration steps (Protocols II and III). In order to confirm that there was no inherent difference between the treatments in Protocols I, II, and III, selected enhancers were tested with more than one protocol. The treatment protocols for the enhancers are listed in Table 1.

Equilibration With 4 mL of PBS in Diffusion Cell (Protocol II)—Protocol II followed the same 20-min enhancer treatment procedure as described in “Examination of HEM dehydration.” In the post-enhancer equilibration step, in which the enhancer-treated HEM was allowed to equilibrate with a total of 4 mL PBS in the diffusion cell, the concentration of the enhancer in the equilibrating PBS solution was determined using HPLC or GC assay. Transport experiments were conducted with the enhancer-PBS solution in the donor and receiver at the enhancer concentration determined in the assay. In the cases where the concentration of the enhancer was below detection limit, PBS alone was used as the vehicle in the transport experiments.

Equilibration With 0.5 mL of PBS in Scintillation Vial (Protocol III)—In Protocol III, HEM was treated following the procedure as stated in “Equilibration With 4 mL of PBS in Diffusion Cell” except that the post-enhancer equilibration step was modified. This modification was based on enhancer depletion that was anticipated when 4 mL PBS were used. After the treatment with the PBS-saturated enhancer, the enhancer-treated HEM was placed in a scintillation vial containing 0.5 mL of PBS, tilted at a 45° angle and placed in a shaking water bath at 37°C for 2 h. Fifty microliters of the equilibrating PBS were then analyzed by HPLC or GC and the concentration of the enhancer in PBS was determined. Enhancer solution was then prepared at this concentration in PBS and was used in the donor and receiver in the transport experiments. Preliminary calculations using Eq. (5) suggest that the depletion of the enhancer in HEM would be minimal when 0.5 mL of PBS was used:

$$K_{\text{oct}} = \frac{X_{\text{L,enhancer}} / V_{\text{Lipid}}}{X_{\text{PBS,enhancer}} / V_{\text{PBS}}} \quad (5)$$

where $X_{\text{L,enhancer}}$ is the amount of enhancer in the lipid domain, V_{Lipid} the volume of the lipid which is assumed to constitute 20% of total SC volume, $X_{\text{PBS,enhancer}}$ the amount of the enhancer in the equilibrating PBS and V_{PBS} the volume of equilibrating PBS solution.

Enhancer Depletion and Recovery of HEM Barrier

The recovery of HEM after enhancer treatment was studied and possible irreversible changes such as lipid extraction of HEM was examined. The question of how long the less lipophilic enhancers (with $\log K_{\text{oct}} < 4.5$) would be able to sustain their enhancement effects on HEM in PBS was examined.

HEM samples were prepared, treated with PBS-saturated enhancers for 20 min, and mounted in side-by-side diffusion cells as described in Protocol II. PHE, OC, and OP were the enhancers studied. After the 2 h of PBS equilibration in the post-enhancer equilibration step, PBS in both chambers was replaced with fresh PBS and the donor sample was spiked with ^3H -CS. Transport experiments were carried out as stated in “HEM Transport Experiments: PBS/Control” using PBS as the vehicle.

Silicone Elastomer Uptake

Silicone elastomer samples weighing 7.0 ± 2.5 mg were placed in scintillation vials with 2 mL of the enhancer or 2 mL PBS saturated with the enhancer. The vials were kept in a shaking water bath at $25 \pm 1^\circ\text{C}$ for 48 h. Silicone elastomers were then removed and washed with distilled deionized water three times and patted dry with Kimwipes. The washed silicone elastomers were then placed in scintillation vials containing 5 mL of ethanol, which were then shaken at 25°C for another 48 h. Aliquots of the ethanol were analyzed using HPLC or GC to determine the concentration of enhancer in the silicone elastomer. The silicone elastomer was exposed to a second extraction using 2 mL of ethanol for 24 h to ensure total extraction of

enhancer from silicone elastomer. A third extraction again with 2 mL of ethanol was carried out when the second extraction showed more than 10% of the enhancer in the first extraction.

HPLC Assay

The HPLC system was consisted of SIL-20A (total-volume injection type autosampler) auto-sampler, SPD-20A Prominence UV/VIS detector, LC-20AT pump (Shimadzu Scientific Instruments, Columbia, MD), and a 15 cm long, 4.6 mm diameter Microsorb C18 column purchased from Varian, Inc. (Palo Alto, CA). The detection wavelengths, flow rates, and mobile phases were: 220 nm, 1 mL/min, 70% methanol in water for HP; 298 nm, 1 mL/min, 75% methanol in water for BHA; 220 nm, 1 mL/min, 80% methanol in water for OP; 307 nm, 1 mL/min, 90% methanol in water for OS; 266 nm, 0.5 mL/min, 6.5% water and 0.5% triethyl amine in methanol for PHE;³⁵ 254 nm, 1.3 mL/min, 60% acetonitrile in water for SA; 310 nm, 1 mL/min, 80% acetonitrile in water for PadO; 220 nm, 2 mL/min, 85% acetonitrile in water for DoP, 248 nm, 1 mL/min, 65% methanol in water for CS, respectively. For all the HPLC analyses performed, the calibration curves were constructed based on the peak areas with standard solutions prepared in methanol.

GC Assay

GC analyses were performed on a Shimadzu GC-2014 fitted with a flame ionization detector (FID) and AOC-20i auto injector. The capillary column was 15 m in length, 0.25 mm inner diameter, and with film thickness of 0.25 μ m. All the analyses were performed using helium as the carrier gas at a total flow-rate of 1.57 mL/min. The samples were injected using a split mode with split ratio of 20:1. The injector temperature, FID detector temperature, and column oven temperature were: 250°C, 250°C, 90°C for 1 min then to 210°C at a rate of 20°C/min and held at 210°C for 4 min for OL; 250°C, 250°C, 120 to 220°C at a rate of 20°C/min for OC; 250°C, 250°C, 150°C for 1 min to 250°C at a rate of 10°C/min for IPM; 300°C, 400°C, 250°C for 1 min to 380°C at a rate of 20°C/min for AZ; 250°C, 250°C, 70 to 200°C at a rate of 15°C/min and held at 200°C for 1 min for HL; 320°C, 320°C, 180 to 320°C at a rate of 30°C/min for OA; 300°C, 300°C, 180 to 280°C at a rate of 15°C/min for UD; 250°C, 250°C, 80°C for 1 min to 200°C at a rate of 15°C/min for BA; 250°C, 250°C, 100 to 200°C at a rate of 10°C/min for ME, respectively. For all the GC analyses performed, the calibration curves were constructed based on the peak areas with standard solutions prepared in methanol.

RESULTS AND DISCUSSION

HEM Transport Experiments and Enhancement Factor Determination

The permeability coefficients of CS in the absence of the enhancers (Tab. 3) were determined on thirteen different skin donors in the control experiments, and these results were consistent with those reported previously.³⁴ The aqueous solubilities of the enhancers in PBS are shown in Column 5 of Table 2. The CS solubility ratios, determined in the CS solubility experiments, are presented in Column 4 of Table 3. These solubility ratios were used in the calculations of Eq. (3). For the enhancers in which the enhancer aqueous solubilities were below the detection limit in the HPLC and GC assay, the calculated aqueous solubilities from EPI suite (EPA database) was used, and the CS solubility ratios were assumed to be unity. The enhancement factors of CS transport across HEM were then calculated as described in Eq. (3) using the permeability coefficients and solubility ratios.

Optimization of Direct Enhancer Treatment

To optimize the enhancer treatment procedure in Protocols II and III, the effects of enhancer treatment times were studied. Figure 3 shows the permeability coefficients of HEM for CS after direct enhancer treatments of HEM with OS, PADO, IPM, UD, and OA for 1 min, 4 min,

20 min, and 12 h. The treatment times tested in the present study did not significantly affect the permeability coefficients of CS ($p > 0.05$, t -test), indicating that the partitioning of these enhancers into HEM was fast. The rapid enhancer uptake into HEM in the 20-min enhancer treatments is consistent with the relatively short transport lag time of moderately lipophilic permeants in enhanced transdermal permeation.³⁶ The absence of statistical differences between the enhancement results after the seemingly insufficient 1 and 4 min treatments versus the 20 min treatments could be attributed to the 2-h equilibration before the transport runs that would allow the enhancers in other HEM domains to redistribute into the SC lipids. When the enhancer treatment time was extended to 12 h, there was a significant decrease in CS permeability coefficients compared to those with the shorter treatment times (1, 4, and 20 min). It was hypothesized that the decrease in the permeability coefficients was a result of HEM dehydration³⁷ after the prolonged enhancer treatment. This hypothesis was supported by the results of the 12-h treatment study using enhancers saturated with PBS, in which the permeability coefficients were not significantly different from those with the shorter treatment times ($p > 0.05$, t -test). The data also suggest that HEM dehydration was not significant with the shorter treatment such as in the 20-min OS treatment with and without PBS saturation ($p = 0.3$, t -test, data not shown).

Based on the above findings, direct enhancer treatments (Protocols II and III) were conducted for 20 min to minimize the possibility of lipid extraction that might occur using the longer treatment times, even though the present results did not suggest significant lipid extraction in the 12-h treatment. To minimize the impact of dehydration, the enhancers used were saturated with PBS.

Comparison of Enhancer Treatment in Protocols I, II, and III

To verify that the different enhancer treatment protocols provided the same penetration enhancement in HEM, selected enhancers (based on the physicochemical properties of the enhancers) were studied under two different protocols: Protocols I and III or Protocols II and III. PADO and IPM were examined in both Protocols II and III, and OP, OC, and ME were examined in Protocols I and III. Table 3 summarizes the permeability coefficients of HEM for CS and the enhancement factors in these studies. No significant difference was observed between the enhancement factors in Protocols II and III ($p = 0.31$ and 0.23 for PADO and IPM, respectively, t -test) and in Protocols I and III ($p = 0.4$, 0.27 , and 0.09 for OP, OC, and ME, respectively, t -test). The essentially same enhancement factors with the respective enhancers under the different protocols suggest that Protocols I, II, and III essentially lead to the same HEM enhancement conditions. This allowed the direct comparison of the enhancer results in the present study even when the enhancers were examined under the different protocols (Protocols I, II, and III).

HEM Electrical Resistance and the Pore Pathway

In Protocol I, the electrical resistances of HEM after the enhancer treatments were found to be within 30% of their respective initial resistances. The changes in HEM electrical resistance due to the enhancer treatments were minor in comparison to the enhancement in CS permeability across HEM. This suggests that the flux enhancement was mainly a result of the changes in the lipoidal pathway rather than the pore pathway of the membrane. In Protocols II and III, the initial electrical resistances of HEM were not determined. HEM resistances were determined in these two protocols only after the direct enhancer treatments and at the completion of the transport experiments. In all the experiments, HEM resistances were greater than $10 \text{ k}\Omega\text{cm}^2$, indicating that the integrity of the HEM pore pathway was not compromised. The electrical resistances after the transport experiments were also found not to be significantly different from those determined before the transport experiments, indicating that the integrity of HEM was maintained during the experiments. It is interesting to point out that no relationship

between the electrical resistance of HEM and its permeability coefficient for CS was observed in the present study (data not shown). This is consistent with the hypotheses that the SC lipid domain is the predominant permeation pathway of CS across HEM^{31,34,38} and that the enhancers mainly affect this lipoidal pathway rather than the pore pathway in HEM.

Relationship Between $E_{\max}$ and $K_{\text{oct}} \times S_w$

Previous studies have suggested a relationship between the enhancement effects of permeation enhancers and the enhancer concentration in SC.^{21,34,39} It has also been suggested that the site of action of enhancers in the lipid domain is a semipolar microenvironment that resembles the properties of *n*-alkanols such as *n*-octanol.^{21,24} The ability of an enhancer to exert its enhancement effect is related to its ability to partition and translocate into this semipolar region within the SC. To this end, a possible relationship between $E_{\max}$ (i.e., the enhancer potency) and the product of K_{oct} (octanol water partition coefficient) and S_w (aqueous solubility of enhancer) was examined (Fig. 4). The product $K_{\text{oct}} \times S_w$ is the hypothetical enhancer solubility in *n*-octanol that resembles the solubility of the enhancers in the SC lipid domain. The figure shows a trend of increasing $E_{\max}$ with an increase in $K_{\text{oct}} \times S_w$, consistent with the hypothesis that the enhancer concentration in the lipid domain is a key factor to transdermal permeation enhancement. Under this hypothesis, because the potency of a chemical enhancer based on $E_{\max}$ is related to its solubility in the lipid domain, the enhancer with a higher thermodynamic energy state in its pure physical form, that is, higher S_w , is expected to be more potent. Figure 4 also shows considerable data scatter due to the uncertainties of the K_{oct} and S_w values from both experimental as well as calculated values in the literature. For example, DoP has higher $E_{\max}$ and SA has lower $E_{\max}$ than predicted from this $E_{\max}$ versus $K_{\text{oct}} \times S_w$ relationship.

Depletion of the Enhancer in HEM and HEM Recovery

The purposes of this study were to determine (a) the reversibility of the enhancer effects and the ability of HEM to recover after the enhancer treatment and (b) the duration of the flux enhancing effect of these enhancers when enhancer depletion in HEM occurred. In this study, the recovery of HEM barrier after the treatment of PHE, OC, and OP was determined using Protocol II. Complete recovery of HEM after enhancer treatment was observed with these enhancers (Tab. 4), indicating that the treatment of the enhancers did not lead to irreversible alteration of HEM. This suggests that the enhancers did not extract lipids from the SC during enhancer treatment or that the lipids extracted during the treatment had no impact on the SC lipoidal transport pathway.

It was hypothesized that the depletion of the relatively hydrophilic enhancers (compared to the other enhancers in the present study) from HEM might limit the effectiveness of these enhancers in practice *in vivo*. For PHE, the recovery of skin occurred in less than 2 h in the equilibration period before the start of the transport experiments. For OC and OP, having higher K_{oct} and lower aqueous solubility than PHE, the permeability coefficients of HEM returned to the PBS control value in approximately 6 h, possibly due to slower depletion (data not shown). This suggests that the more lipophilic enhancers are likely to retain their transdermal enhancement effects due to slower enhancer depletion when the source of the enhancers is removed from the skin *in vivo*. For the very lipophilic enhancers OS, PADO, IPM, UD, and OA studied under Protocol II, no effects attributed to enhancer depletion were observed within the duration of the transport experiments in the present study.

Silicone Uptake Data

The purpose of the silicone uptake study was to compare the hypothetical solubilities of these enhancers in octanol and in silicone elastomer to mimic the microenvironment of the SC lipid domain. The last column in Table 2 shows the concentration of the enhancers in the silicone elastomer at saturation. In all the silicone elastomer studies, the second extraction of the

enhancers from the elastomer was less than 10% of the first extraction, suggesting that the first extraction was adequate. Figure 5 is a plot of the maximum enhancement factor $E_{\max}$ against the solubility of the enhancers in silicone elastomer ($r^2 = 0.57$). It is evident from the figure that a trend exists between $E_{\max}$ and the solubilities of the enhancers in the silicone elastomer. The enhancement factor increases with the enhancer solubility in the elastomer similar to the results in Figure 4. This suggests that the silicone elastomer can be used to predict $E_{\max}$.

Structure Activity Relationship in Transdermal Enhancement: $E_{\max}$ Versus K_{oct}

The ability to select a good penetration enhancer to improve transdermal delivery relies on the understanding of the enhancer structure-activity relationship. Since the enhancer treatments in the present study would result in the enhancers in the SC lipid domain at their highest thermodynamic activities in equilibrium, the enhancement effects reported here represent the highest enhancement induced by these compounds alone in SC. Figure 6 shows the relationship between the potencies or maximum enhancement factors of the enhancers ($E_{\max}$) versus their lipophilicities or octanol/water partition coefficients (K_{oct}). From the results, the studied enhancers can be divided into two main groups: (a) long hydrocarbon chain (≥ 6) enhancers, HL, OC, OP, DoP, HP, OA, UD, OA, OS, OL and IPM (open symbols, crosses, and dashes in Fig. 6) and (b) enhancers with a cyclic or compact hydrocarbon moiety and do not have long hydrocarbon chain, PHE, BHA, BA, SA, ME, and PADO (closed symbols in Fig. 6). In general, both groups demonstrate similar $E_{\max}$ versus K_{oct} relationships of decreasing $E_{\max}$ with increasing K_{oct} of the enhancers. This is an interesting finding as one would intuitively expect that the more lipophilic enhancers are likely to be more potent and would have higher $E_{\max}$ values. The general correlation between $E_{\max}$ and K_{oct} , relatively independent of enhancer chemical classes, suggests no specific interaction between the different moieties of the enhancers and SC lipids in transdermal permeation enhancement, which is consistent with the previous finding that the polar head groups of the alkyl-chain enhancers only assist in translocating the enhancers to and aligning the enhancers within SC lipids.^{23,24} Although the second group of enhancers shows a similar trend of $E_{\max}$ versus K_{oct} relationship as the first group, the potencies of these enhancers tend to be lower than the enhancers in the first group (Fig. 6). Possible explanations of the lower enhancement potencies of the second group enhancers in comparison to the first group could be (a) the lower effectiveness of the compact enhancers to fluidize the SC lipids and/or (b) lower solubilities and concentrations of these enhancers in the SC lipid domain. The later is a more likely explanation because the cyclic or compact enhancers (closed symbols) do not deviate from the general $E_{\max}$ versus $K_{\text{oct}} \times S_w$ relationship in Figure 4.

$E_{\max}$ and Transdermal Drug Delivery

The present study focused on understanding the maximum enhancement effect ($E_{\max}$) of a number of commonly known cosmetic and topical pharmaceutical ingredients. The objective of this direct enhancer treatment was twofold. First, this method allowed the examination of $E_{\max}$ induced by these ingredients; this method could overcome the limitations accompanying the study of the highly lipophilic enhancers as pointed out in the Introduction Section.²⁸ Second, the method was similar to the conditions in a transdermal aerosol system where the solvent would evaporate in the process. Topical spray formulations are mainly composed of the drug, a volatile solvent, and a permeation enhancer. In the aerosol system, drug permeation enhancement is mainly attributed to the chemical enhancer alone. The direct exposure of the chemical enhancer on skin is therefore closely mimicked by the direct treatment of skin with neat enhancer when the enhancer exerts its maximum enhancement effect in the absence of any solvents or co-solvents.

A major finding in the present study was that BA, PHE, and BHA are relatively potent enhancers. These compounds are used as preservatives in topical products and have never been

mechanistically studied for their penetration enhancement effect. Also, these cosmetic ingredients were found to be as potent as (or more potent than) some of the common enhancers (e.g., OA and AZ). Previous studies have attempted to identify potent enhancers and to establish a predictive method for enhancer potency based on the physicochemical properties of the enhancers.¹⁹ OS and PADO have been used as enhancers in topical spray to enhance transdermal penetration of hormones.^{29,30} Laurocapram (AZ) and its derivatives have been extensively studied for their penetration enhancement effects.^{40–42} IPM⁴³ and OL⁴⁴ have also been studied for their enhancement on skin permeability. It has been previously suggested that there is an initial increase in enhancer potency with an increase in enhancer alkyl chain length up to C9–12 and beyond which a decrease in the potency of enhancers occurs.^{45–47} It should be pointed out that co-solvents were used in most transdermal chemical enhancer studies in the literature, and possible synergistic effects between the enhancer and co-solvent were not completely understood. For example, co-solvents can alter the thermodynamic activities of the enhancers in formulations, resulting in a change in the partitioning of the enhancers into SC. It has also been shown that the same enhancer in different co-solvent systems can result in opposing effects of enhancement or retardation on skin.⁴⁸ As a result, these previous studies can only identify the enhancer effects under the particular experimental conditions in the studies. In the present study, the use of co-solvents or solubilizing agents was avoided.

Selection of Enhancers for Transdermal Products

According to the findings in the present study, the enhancers can be divided into three categories: low, moderate, and highly lipophilic enhancers ($\log K_{\text{oct}} < 3$, $\log K_{\text{oct}}$ between 3 and 5, $\log K_{\text{oct}} > 5$, respectively). The enhancers of low lipophilicity provide a high degree of enhancement (based on the enhancer maximum effect) but enhancer depletion can be a problem. As shown in the depletion study, enhancer clearance from the skin would result in a decrease in the enhancement effect. The continuous depletion of the enhancer from the skin into the body would also create an enhancer concentration gradient within the SC and can affect penetration enhancement. The enhancers of moderate lipophilicity induce lower transdermal enhancement than the low lipophilic enhancers but are expected to retain their effects for a longer duration. The higher lipophilic enhancers provide the lowest enhancement, yet their enhancement effects are retained over the duration of the present study. The proper selection of enhancers in transdermal drug delivery would depend not only on the enhancement factor achieved but also the retention of the enhancer effects in SC. For example, the medium alkyl-chain enhancers with moderate lipophilicity would be better enhancers in a transdermal spray product. In addition, independent of the enhancer lipophilicities, the alkyl-chain enhancers were observed to be more effective than the nonalkyl chain enhancers in the present study.

It should be noted that the selection of proper chemical penetration enhancers for transdermal products also requires the assessment of the biological effects of the enhancers such as skin irritancy. These effects are more difficult to predict from the physicochemical properties of the enhancers. The present paper has investigated mainly the chemical enhancers that are commonly used in cosmetic and transdermal products and are classified as safe or are FDA approved. In summary, the present study has shown that E_{max} decreases with an increase in enhancer lipophilicity. Despite the higher E_{max} of the lower lipophilic enhancers, depletion of the enhancers from SC such as in a transdermal aerosol system *in vivo* may limit their use as transdermal penetration enhancers.

CONCLUSION

The enhancement effects of a wide variety of compounds in different chemical classes on transdermal permeation were investigated. A relationship between the maximum intrinsic enhancement factor (E_{max}) and enhancer lipophilicity (K_{oct}) was observed with the enhancers,

in which the enhancer potency decreased with increasing enhancer lipophilicity. The general $E_{\max}$ versus K_{oct} relationship suggests that the potency of an enhancer is relatively independent of specific interactions between the enhancer and SC lipids. The results also suggest that the solubility of the enhancer in SC is an important factor for transdermal transport enhancement. The $E_{\max}$ versus $(K_{\text{oct}} \times S_w)$ relationship is consistent with the previous hypothesis that permeation enhancement is related to the enhancer concentration in SC lipids. Enhancer depletion from skin, which is related to the lipophilicity of the enhancer, could be another important factor in the selection of suitable enhancers for transdermal drug delivery. For the enhancers examined in the present study, the compounds commonly used in topical products were found to have similar or higher apparent $E_{\max}$ than some of the well-known enhancers. $E_{\max}$ of the nonalkyl chain enhancers was found to be lower than that of the alkyl-chain enhancers when compared at the same lipophilicity. This study also proposed the possibility of using enhancer solubility in silicone as a predictive tool for determining the potency of an enhancer.

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Figure 1.
Structural formulas and abbreviations of the chemicals examined in the present study.

Figure 2. Experimental design and the selection of the proper protocol based on enhancer $\log K_{oct}$.

Figure 3. Effects of enhancer treatment time and PBS saturation upon the permeability coefficients of CS with enhancers OS, PADO, IPM, UD, and OA. $n \geq 3$ with at least three different skin donors.

Figure 4.
 E_{max} versus the product of $K_{oct} \times S_w$ (Molar) (the hypothetical solubility of the enhancers in octanol).

Figure 5.
 E_{max} versus saturated enhancer concentrations in the silicone polymer (moles/mg of silicone) in the silicone uptake study.

Figure 6.

Relationships between E_{max} and the $\log K_{oct}$ of the enhancers. For OL, $\log D$ instead of $\log K_{oct}$ was used. Open symbols including crosses and dashes: enhancers with long hydrocarbon chain (≥ 6); closed symbols: enhancers with a cyclic or compact hydrocarbon moiety and do not have long hydrocarbon chain. Mean $\pm$ SD ($n \geq 3$) with at least three different skin donors. The error bar of BHA is not shown because it lies within the symbol.

Table 1

A Summary of the Enhancers Used in the Present Study and the Treatment Protocol Used in the Transport Studies

Enhancer ^a	Product Information/Regulation and Safety/References	Treatment Protocol		
		I	II	III
OS	Up to 5% (FDA limit for Sunscreens) More than 760 products: e.g., Avance face SPF 20 (ID 19674) Celazome (ID 17320, 17328, 17329) ELTA Swiss American (ID 12650, 12655, 12659) Glymed Plus (ID 19365, 19371, 19383)		*	
PADO	Up to 8% (FDA limit in Sunscreens) More than 147 products: e.g., PADIMATE O lip balm (Herpecin-L [®]) Hawaiian tropic (for kids) Neurogena chemical free sunblocker Herpecin-L-cold sore		*	*
IPM	FDA GRAS EAFUS Low order of acute toxicity More than 799 products: e.g., Dermablend wrinkle fix Hand sense protective skin Yu-Be moisturizing skin Camo care soothing cream Avon moisture therapy oatmeal hand cream		*	*
OL	FDA GRAS EAFUS High concentration may cause skin redness and mild irritation More than 537 products: e.g., Vivelle, Novartis Pharmaceuticals Iodex anti-infective Dove foam conditioner			*
PHE	FDA GRAS EAFUS More than 4355 products: e.g., Hepatitis A vaccine Lidosite Patch L'Oreal sublime glow for face L'Oreal visible results	*		
BHA	FDA GRAS EAFUS In the US, an estimated daily intake of up to 4.3 mg More than 386 products: e.g., Dermatar ointment (0.1%) Euvitol cream (0.2%) Micatin athletes foot cream	*		
UD	Up to 4% in a fragrance concentrate Common fragrance ingredient		*	
OA	FDA GRAS indirect additives used in food contact substances More than 556 products: e.g., Reviva Ahhhloe Ice Massage Combipatch Vitamin shoppe wrinkle relief		*	
OP	A previous study showing its permeation enhancement effect ³⁴	*		*
DoP	FDA GRAS EAFUS More than 49 products: e.g., Final net hair spray Mink difference nonaerosol hair spray Biosilk therapy shampoo			*
HL	Antifoaming, fragrance co-solvent, and viscosity decreasing agent	*		
OC	Study showing its permeation enhancement effect ³⁴	*		*
BA	FDA GRAS EAFUS More than 1392 products: e.g., Lamin hydrating gel ProCytelamin hydrating gel Aveeno daily baby lotion	*		
SA	Flavoring agent in tobacco products (0.01%)	*		
ME	FDA GRAS indirect additives used in food contact substances	*		*
HP	A previous study showing its permeation enhancement effect ²⁸	*		
AZ	Previous studies showing its enhancement effect ^{8,49–51}			*

* Indicates the protocols used for enhancer treatment and transport studies.

^aSee Figure 1.

Table 2

A Summary of the Physicochemical Properties of Enhancers: Molecular Weight of the Enhancers (MW), Logarithm of the Octanol Water Partition Coefficient ($\log K_{\text{Oct}}$), and the Molar Aqueous Solubility of Enhancers (S_w)

Enhancer	Full Chemical Name	MW (g/mol)	$\log K_{\text{Oct}}$	S_w (M) ^e	Silicone Uptake $\times 10^{-7}$ (mol/mg)
OS	2-Ethylhexyl 2-hydroxybenzoate	250.3	5.97 ^a	$2.9 \times 10^{-6} c$	4.8 ± 1.2
PADO	2-Ethylhexyl 4-(dimethylamino) benzoate	277.4	5.76 ^a	$7.1 \times 10^{-7} c$	1.3 ± 0.2
IPM	Propan-2-yl tetradecanoate	270.4	7.3 ^a	$4.9 \times 10^{-8} c$	3.1 ± 0.4
OL	9,10-Octadecenoic acid	282.5	7.64 ^a , $\log D$ 5.06 ^g	$4.07 \times 10^{-8} c$	1.44 ± 0.01
PHE	2-Phenoxy-1-ethanol	138.2	1.16 ^{b52}	0.18 ^d	5.0 ± 1.5
BHA	1,1-Dimethylethyl-4-methoxy phenol	180.2	3.14 ^{b53}	0.008 ^{d,h}	1.4 ± 0.2
UD	Undecan-1-ol	172.2	4.2 ^{b54}	$3 \times 10^{-4} c$	1.9 ± 0.2
OA	(Z)-Octadec-9-en-1-ol	268.4	7.0 ^a	$8.8 \times 10^{-8} c$	0.63 ± 0.04
OP	1-Octyl-2-pyrrolidinone	197.3	3.3 ^a	$4.8 \times 10^{-3} d$	4.5 ± 1.0
DoP	1-Dodecyl-2-pyrrolidinone	253.4	4.2 ^{b55}	$2.1 \times 10^{-5} d$	6.7 ± 1.1
HL	1-Hexanol	102.1	2.03 ^{b56}	0.056 ^d	17 ± 4
OC	1-Octanol	130.2	3.0 ^{b56}	$6.0 \times 10^{-3} d$	8.1 ± 1.4
BA	Benzenealdehyde	108.1	1.06 ^a	0.38 ^d	5.3 ± 1.5
SA	o-Hydroxybenzaldehyde	122.3	2.0 ^{a,g}	0.09 ^d	6.4 ± 1.4
ME	(2S,5R)-2-Isopropyl-5-methylcyclohexanone	154.2	2.87 ^a	0.003 ^{d,h}	4.0 ± 0.7
HP	1-Hexyl-2-pyrrolidinone	169.2	2.35 ^a	0.079 ^d	23 ± 5
AZ	1-Dodecylhexahydro-2H-azepin-2-one	281.4	6.28 ^{b57}	$2.4 \times 10^{-7} c$	3.1 ± 0.4

The last column shows the solubility of the enhancer in silicone elastomer in mol/mg of silicone.

^a Calculated $\log K_{\text{Oct}}$ based on the chemical structure of the compound, obtained from EPI Suite Database.

^b Experimental $\log K_{\text{Oct}}$ from the literature.

^c Calculated water solubility, based on chemical structure of the compound, obtained from EPI Suite Database.

^d Experimental values of aqueous solubility of enhancer at 25°C determined in the present study.

^e $n \geq 3$.

$f_n = 4$.

^gIndicates log octanol/water distribution coefficients ($\log D$) of oleic acid ($pK_a = 5.02$) and SA ($pK_a = 8.4$) at pH 7.4.

^hCompounds that have a number of isomers and the solubilities are of the chemical lots used.

Table 3

Permeability Coefficient of HEM for CS and CS Solubility Ratio in Enhancer/PBS Solution and PBS*

Enhancer	Protocol	Permeability Coefficient of CS for HEM (10^{-7} cm/s)	CS Solubility Ratio	Enhancement Ratio
None	NA	3.7 ± 1.8	NA	NA
OS	II	16 ± 8	1	5.0 ± 3.6
PADO	II	15 ± 2	1	4.1 ± 0.7
	III	13 ± 5	1	3.8 ± 1.1
IPM	II	10.7 ± 2.5	1	2.8 ± 0.2
	III	11 ± 3		3.3 ± 1.1
OL	III	44 ± 19	1	13 ± 2
PHE	I (0.18 M)	23 ± 2	2.45 ± 0.06	12 ± 4
BHA	I (0.008 M)	28 ± 9	1.16 ± 0.03	6.5 ± 0.4
UD	II	49 ± 8	1	17 ± 2
OA	II	16 ± 6	1	5.5 ± 1.5
OP	I (0.0048 M)	55 ± 21	0.93 ± 0.02	18 ± 5
	III (0.0048 M) ^a	50 ± 26		16 ± 6
DoP	III (2.1×10^{-5} M) ^a	47 ± 9	0.98 ± 0.02	14 ± 6
HL	I (0.056 M) ^a	54 ± 9	1.05 ± 0.02	20 ± 2
OC	I (0.006 M)	55 ± 1	1.02 ± 0.02	20 ± 2
	III (0.006 M) ^a	60 ± 14	1.02 ± 0.02	18 ± 2
BA	I (0.38 M)	32 ± 5	1.36 ± 0.06	16 ± 4
SA	I (0.08 M)	13 ± 3	1.74 ± 0.05	8.0 ± 1.1
ME	I (0.0068 M) ^a	30 ± 2	1.04 ± 0.03	11 ± 2
	III (0.005 M)	16 ± 6	1.03 ± 0.02	7.9 ± 1.4
HP	I (0.079 M)	36 ± 8	1.02 ± 0.04	26 ± 5
AZ	III	25 ± 4	1	9 ± 2

The CS solubility ratio for transport experiments where the equilibrating PBS enhancer concentration was below detection limit of the chromatographic method applied for analysis is considered unity.

* $n \geq 3$ with at least three different skin donors.

^a Indicates the concentration of the enhancer found in the equilibrating PBS used in Protocol III.

Table 4

Permeability Coefficient (cm/s) of HEM for CS at Steady-State in Protocol II with Enhancer Depletion and in Protocol I without Depletion

Enhancer	Steady-State Permeability Coefficient (10^{-7} cm/s) Without Enhancer Depletion	Steady-State Permeability Coefficient (10^{-7} cm/s) With Enhancer Depletion
PHE	23 ± 2	5.1 ± 0.1
OC	58 ± 9	5 ± 1
OP	53 ± 21	5.1 ± 0.1