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Evaluation of Transdermal Drug Permeation as Modulated by Lipoderm and Pluronic Lecithin Organogel

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A B S T R A C T

The transdermal delivery of 2 fluorescent probes with similar molecular weight but different lipophilicity, into and through the skin from 2 commercially available transdermal bases, pluronic lecithin organogel, and Lipoderm® has been evaluated. First, in vitro penetration of fluorescein sodium and fluorescein (free acid) through porcine skin was evaluated. Retention and depth distribution profiles in skin were obtained by tape stripping and then followed by optical sectioning using multiphoton microscopy. The results showed that Lipoderm® led to an enhanced penetration of the hydrophilic compound, fluorescein sodium. For the lipophilic compound fluorescein (free acid), Lipoderm® performed similar to pluronic lecithin organogel base, where minimal drug was detected in either receptor phase. The skin retention and depth distribution results also showed that the hydrophilic fluorescein sodium had high skin retention with Lipoderm®, whereas fluorescein (free acid) had very low penetration and retention with increasing skin depth. Moreover, optical sectioning by multiphoton microscopy revealed an uneven distribution of probes across the skin in the x-y plane for both transdermal bases. This work showed that a hydrophilic compound has significantly increased skin penetration and retention when formulated with Lipoderm®, and the skin retention of the probe was the main determinant of its skin flux.

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Introduction

Delivery of active ingredients through the skin has been an exciting and challenging area of pharmaceutical research. The well-known advantages of this route of administration include easy application, avoidance of first-pass metabolism, and improved patient compliances. Meanwhile, increasing demands for customized drug medications has led to an increase in the practice of compounding in pharmacies.[1] An elegant transdermal base should be compatible with a variety of therapeutic agents and actively transport the medication through the skin directly to the site of action. Pluronic lecithin organogel (PLO) has been widely used as a pharmaceutically acceptable transdermal drug delivery system since its introduction in the 1990s.[2-4] PLO is an opaque, biocompatible preparation, commonly composed of phospholipids (soya lecithin), isopropyl palmitate, and an aqueous phase (aqueous solution of 20%-30% of Pluronic F127). In the recent years, Lipoderm® cream has become a popular base for compounding transdermal needs, though its composition are not publicly known; Lipoderm® shows good performance in product stability[1] and in vivo skin absorbance of prazosin[5] and gabapentin.[6] More interestingly, Lipoderm® base outperformed PLO in transdermal delivery of promethazine hydrochloride[7] and ketoprofen[8] through human skin in vitro. In addition, Lipoderm® base has been used to include special combinations of multiple drugs and found to be capable of delivering up to 4 analgesics (i.e., ketamine hydrochloride, gabapentin, clonidine hydrochloride, and baclofen) simultaneously.[9] However, the effects of these 2 commercially available transdermal bases on skin retention and absorption of formulated agents have not yet been systematically compared.

Intact stratum corneum, the outermost layer of the skin, provides the primary barrier to drug penetration into and through the skin. Great efforts have been made in overcoming this barrier to improve percutaneous drug penetration during the past several decades, and the use of chemical penetration enhancers into topical bases has been extensively investigated. Chemical penetration

Abbreviations used: PLO, pluronic lecithin organogel; PBS, phosphate-buffered saline; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; J_{SS}, steady-state flux; MPM, multiphoton microscopy.

Conflicts of interest: The authors declare no competing financial interest.

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enhancers employed in transdermal drug delivery systems offer many advantages including relatively low cost, design flexibility, ease of application, the possibility of self-administration, improved patient compliance, and easy incorporation into formulations.[10,11] These chemical agents enhance skin transport by a variety of complex mechanisms while exerting their effect on drug partitioning and diffusion phases.[12,13] Both PLO and Lipoderm® creams contain a range of chemical enhancers; thus, the performance of these 2 transdermal bases on skin penetration and retention of formulated agents with various lipophilicities should be evaluated, respectively. Moreover, the use of advanced imaging techniques has facilitated our understanding of the extent of molecular penetration into the skin along transport pathways, as evidenced by variations in fluorescence intensity.[14-16] A key component of this study was observing the depth distribution of fluorescent probes in skin samples by optical sectioning using multiphoton microscopy (MPM).

It is generally believed that the free acid or free base of the drug should be used for transdermal delivery; however, studies[17,18] have suggested that this premise was questionable. Thus, the present study evaluated the functionality of Lipoderm® and PLO on the skin permeation of 2 probes: the sodium salt of fluorescein and its free acid. Flux and diffusivity of probes across excised porcine skin were generated from standard permeation experiments using Franz cells. The stratum corneum was collected by tape stripping, and the compound in each tape and stripped skin was also quantified. Depth distribution in the skin was then visualized by MPM. The selected fluorescent probes were fluorescein sodium ($\log p = -1.52$, molecular weight = 376.27), as a model for hydrophilic compounds, and fluorescein (free acid, $\log p = 3.4$, molecular weight = 332.31) as a model for lipophilic compounds.

Experimental Section

Materials

Fluorescein sodium (98.5%-100.5%), fluorescein (free acid, >95%), phosphate-buffered saline sachets, bovine serum albumin, propylene glycol, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). PCCA Lipoderm® base cream, diethylene glycol monoethyl ether, and excipients to make the PLO including lecithin/isopropyl palmitate solution, potassium sorbate, Pluronic F127 were supplied by a local pharmacy. Methanol (99.8%) was supplied by Merck (LiChrosolv®; Merck, Frenchs Forest NSW, Australia). High-purity (Milli-Q) water was used throughout the study.

Analytical Method

Fluorescein sodium and fluorescein (free acid) concentrations were determined using fluorescence spectroscopy.[19,20] Three replicates of 100 μL for each sample were pipetted into a black 96-well plate, and fluorescence was measured in a microplate reader (PerkinElmer Victor 3; PerkinElmer, Waltham, MA) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. The calibration curve for both probes was in the range of 0.02-5 μg/mL. The limit of detection and quantification was found to be 5 and 20 ng/mL, respectively.

Formulation Preparation

The blank PLO base was prepared as described elsewhere[4]: 20% pluronic gel was first prepared by adding Pluronic F127 and potassium sorbate into Milli-Q water (cooled to 4°C) with continuous agitation for 30 min, and then refrigerated at 4°C until a clear solution was obtained. PLO was made by mixing lecithin/isopropyl palmitate solution and 20% pluronic gel in a ratio of 1:4.

PCCA Lipoderm® base is a blank cream provided ready to use; therefore, no preparation was required.

Pilot studies were conducted to screen for a representative control vehicle and an appropriate probe concentration. Selection criteria were probe could dissolve in the vehicle, and there was positive penetration through the skin at least in 24 h. Finally, 80% DMSO was used as a positive control vehicle in this study. The drug loading of fluorescent probes in all formulations was 10%. The PLO with probes was prepared by loading fluorescein sodium or fluorescein (free acid) into the preweighed blank base, and then vigorously homogenizing by hand with a mortar and pestle. In the case of Lipoderm® cream, fluorescein sodium or fluorescein (free acid) was added to Lipoderm® base along with 10% propylene glycol as a wetting agent. All formulations were kept at room temperature before application and used within 2 weeks. The pH of blank 80% DMSO, cream bases, drug-loaded 80% DMSO, and creams was also measured by using a Mettler Toledo pH meter coupled with an Orion Micro pH electrode (Thermo Fisher Scientific, Scoresby VIC, Australia).

Stability Test

Stability testing was performed to monitor if these preparations remain stable during the whole course of experiments. Thus, conditions including samples left on the bench at room temperature up to 4 weeks and samples applied to skin for 30 h at 32°C were tested. In detail, 2 batches of each formulation were stored in amber-colored glass bottles in triplicate at room temperature up to 4 weeks and at 32°C (in a water bath) up to 30 h, respectively. Samples were examined by observation for any phase separation, change of color, and odor. In addition, probe concentrations were measured using fluorescence spectroscopy to monitor their chemical stability. The extraction solvent was 80% methanol.

In Vitro Skin Permeation Study

Porcine ear skin has developed as an accepted substitute for human skin.[21,22] Ears of large white pigs were collected from the South Australian Health and Medical Research Institute laboratories (Adelaide, SA, Australia) and used as an ex situ skin model. Full thickness skin (800 ± 50 μm) was prepared by carefully removing excess hair and adipose tissue. The skin was stored at −20°C and thawed slowly at 4°C before cleaning with water. The skin integrity was assessed by measuring electrical resistance as described in a previous study.[23]

The skin sample was clamped in a vertical Franz cell (PermeGear, Hellertown, PA) with a diffusion area of 0.785 cm^2, and the receptor chamber was filled with 5 mL of 4% bovine serum albumin in phosphate-buffered saline to ensure sink conditions.[24,25] The receptor fluid was kept at 32 ± 1°C by a water jacket and continuously mixed with a magnetic stirring bar. A 100-mg quantity of each formulation was applied to the skin surface, and a glass rod was used to spread the formulation evenly across the diffusion area. The donor chamber was sealed using Parafilm M (Bemis).

A 500 μL sample was drawn at 2, 4, 6, 8, 12, 24, and 28 h through the sampling port and was replaced with fresh receptor medium. Samples were either stored in a refrigerator and analyzed within 2 days or frozen at −20°C for subsequent high-performance liquid chromatography analysis, within 1 month of sampling. On the day of analysis, frozen samples were thawed to room temperature, and 100 μL of the sample was pipetted into a black 96-well plate for assay. For samples with a detectable but unquantifiable

concentration of probe, 300 μL was taken and mixed with 100 μL of methanol. Three hundred fifty microliters of supernatant was collected and evaporated to dryness (nitrogen gas, 45°C) and redissolved in 100 μL of methanol.

Skin Retention

After collection of the receptor fluid at the last time point, the diffusion cells were disassembled, and excess formulation on the skin was removed with a wet tissue. The skin was cleaned with a mild liquid soap solution (Tork, Clayton South, VIC, Australia) using 2 cotton swabs, rinsed under running tap water for 5 s, and then blotted dry with a tissue.

The stratum corneum at the treated site was removed by sequentially tape stripping 20 times using D-Squame® sampling discs (CuDerm Co.) as reported previously.[26] The mass of stratum corneum removed on each tape was determined by weighing the tapes before and after application to the skin surface. After weighing, the discs and the stripped skin were placed into vials individually, and fluorescein probes were extracted with 0.6 mL of 80% methanol with gentle agitation for 24 h. Methanolic solutions of the extracted blank tapes were used as controls for the tape-stripping samples.

Depth Distribution by Multiphoton Microscopy

Skin samples for microscopy imaging were prepared in the same manner as for in vitro Franz cell penetration studies. Negative control samples were prepared by using the blank vehicle only on the stratum corneum side. After cleaning the skin surface, each skin sample was placed onto a microscope slide with 20 μL saline on the stratum corneum surface and protected with a coverslip.

The distribution of florescent probe in the stratum corneum was examined with a MPM system (Zeiss LSM710; Carl Zeiss Micro-Imaging GmbH, Jena, Germany), coupled to a MaiTai laser (Spectra Physics, Mountain View, CA). A Plan-Apochromat 63X/1.40 oil objective (Carl Zeiss Micro-Imaging GmbH) was used for the acquisition of all images. The samples were excited at 800 nm, and fluorescence signals were recorded from 450 to 620 nm. Z stack images were obtained every 2 μm from the skin surface.

Data Analysis

The cumulative amount (Q) of probe that permeated through the skin into the receptor medium was calculated at each sampling time. For an infinite-dose application, the flux (steady-state flux [J_{SS}]) of solutes through the epidermis was determined from the steady-state portion of cumulative receptor concentration-time plots.[23]

All data were analyzed using Graph Pad Prism 6 (GraphPad Software Inc., La Jolla, CA). Data sets were expressed as mean values ± SD. Student t-test (paired) and 1-way ANOVA with Tukey's test were used to determine the level of significance where applicable. The value of $p \leq 0.05$ was considered as a significant difference.

Results

Formulation Characteristics and Stability

Blank PLO base appears opaque and yellow due to the incorporation of soybean lecithin. It is an emulsion and feels soft, similar to a gel cream. The preparation was easily made, and no heating was required, which is advantageous in loading heat-sensitive agents at room temperature or 4°C, such as peptides and natural ingredients. Lipoderm® base is a white cream with no added fragrance. Probes were mixed into the bases, and all preparations spread readily when tested on skin. The hydrophilic 10% fluorescein sodium dissolved fully in both bases, but the hydrophobic fluorescein in the form of free acid reached its maximum solubility, but dispersed well into the bases after milling in a mortar. During the 4 weeks after preparation, all formulations showed no physical separation or notable change of odor or color when kept at room temperature.

The pH of blank 80% DMSO was 9.2 ± 0.1, whereas the pH of 80% DMSO solution containing 10% fluorescein free acid and sodium salt was 7.2 ± 0.1 and 11.3 ± 0.1, respectively. The pH of PLO and Lipoderm® bases was 6.1 ± 0.1 and 5.2 ± 0.1, respectively. With addition of 10% fluorescein (free acid), the pH was slightly changed to 6.2 ± 0.1 and 5.5 ± 0.1, respectively. However, with addition of 10% fluorescein sodium, the pH of both probe-loaded creams was changed to 8.0-8.3.

The chemical stability of both probes in each formulation was also evaluated. Probe percentages remaining in these bases after 4 weeks were in the range of 98.8%-101.5%, which suggests that good stability for these probes was achieved in 80% DMSO, PLO, and Lipoderm® bases. In addition, no significant loss was found in the formulation samples which were kept at 32°C (in a water bath) up to 30 h.

Skin Penetration Studies

The cumulative amount of permeation of the 2 probes through skin over time is shown in Figure 1. The corresponding *Jss* after completion of the in vitro Franz cell study is shown in Table 1. It is evident that the skin penetration of fluorescein sodium was greatly enhanced by Lipoderm® base compared to PLO base and to the

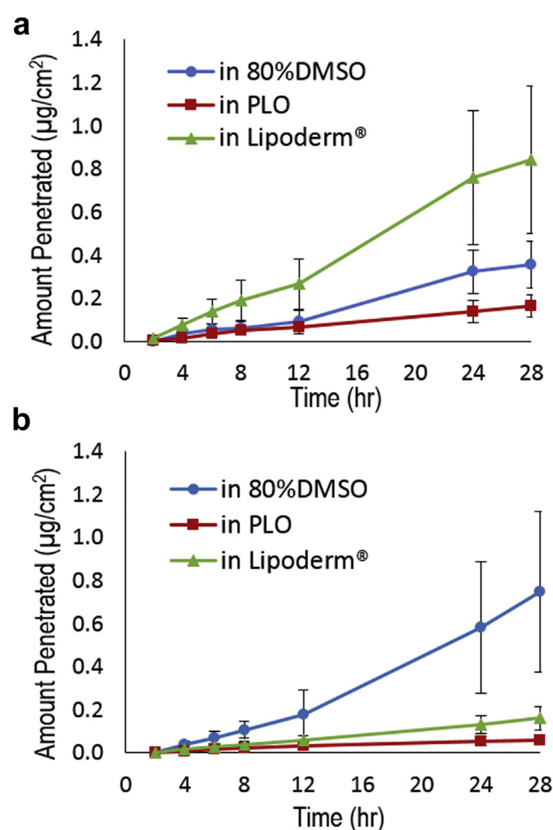

Figure 1. Skin permeation profiles of fluorescein sodium (a), and fluorescein (free acid) (b) from different formulations (mean ± SD, $n = 6$). Probe concentration in all formulations was 10% (w/w).

Table 1
Skin Steady-State Fluxes (*Jss*) of 2 Probes From 3 Formulations After 28 h permeation Study Through Porcine Ear Skin

Solute	*Jss* ($ng/cm^2/h$, Mean ± SD, $n = 6$)		
	80% DMSO	PLO	Lipoderm®
Fluorescein sodium	15.3 ± 5.2	6.1 ± 1.9	33.6 ± 13.6
Fluorescein (free acid)	32.3 ± 16.7	2.1 ± 0.7	6.0 ± 2.2

control formulation, (i.e., 80% DMSO as the vehicle). The total amount of fluorescein sodium that penetrated through the skin from the 80% DMSO was greater compared to PLO base; however, the difference was not significant ($p = 0.2$).

In contrast, the lipophilic fluorescein (i.e., free acid) in 80% DMSO, PLO, and Lipoderm® bases showed much lower skin penetration profiles in comparison with fluorescein sodium. Among these 3 formulations, 80% DMSO gave the maximum permeation. Only minimal concentrations could be detected in the receptor medium when PLO or Lipoderm® was used as the base. Multiple comparisons using Tukey's test showed that there was no significant difference in the mean total amount of fluorescein (free acid) that permeated through the skin and the corresponding *Jss* between PLO and Lipoderm® bases ($p = 0.7$).

Skin Retention

Probe quantities were recovered from each tape strip collected at the end of the skin penetration study with the different formulations applied. In general, the recovered amount of both probes from each of the formulations gradually reduced with increase in tape strip number, corresponding to greater depth into the stratum corneum. To compensate the difference in stratum corneum removal by each tape strip, the stratum corneum thickness as removed by tapes was calculated by using the weight difference of the tape strip and assuming stratum conreum with a density of 1 g/cm^3.[27,28] For fluorescein sodium, (as shown in Fig. 2a), distribution profiling in the stratum corneum was quite similar between 80% DMSO and Lipoderm® as the vehicle. Fluorescein (free acid) from 80% DMSO also

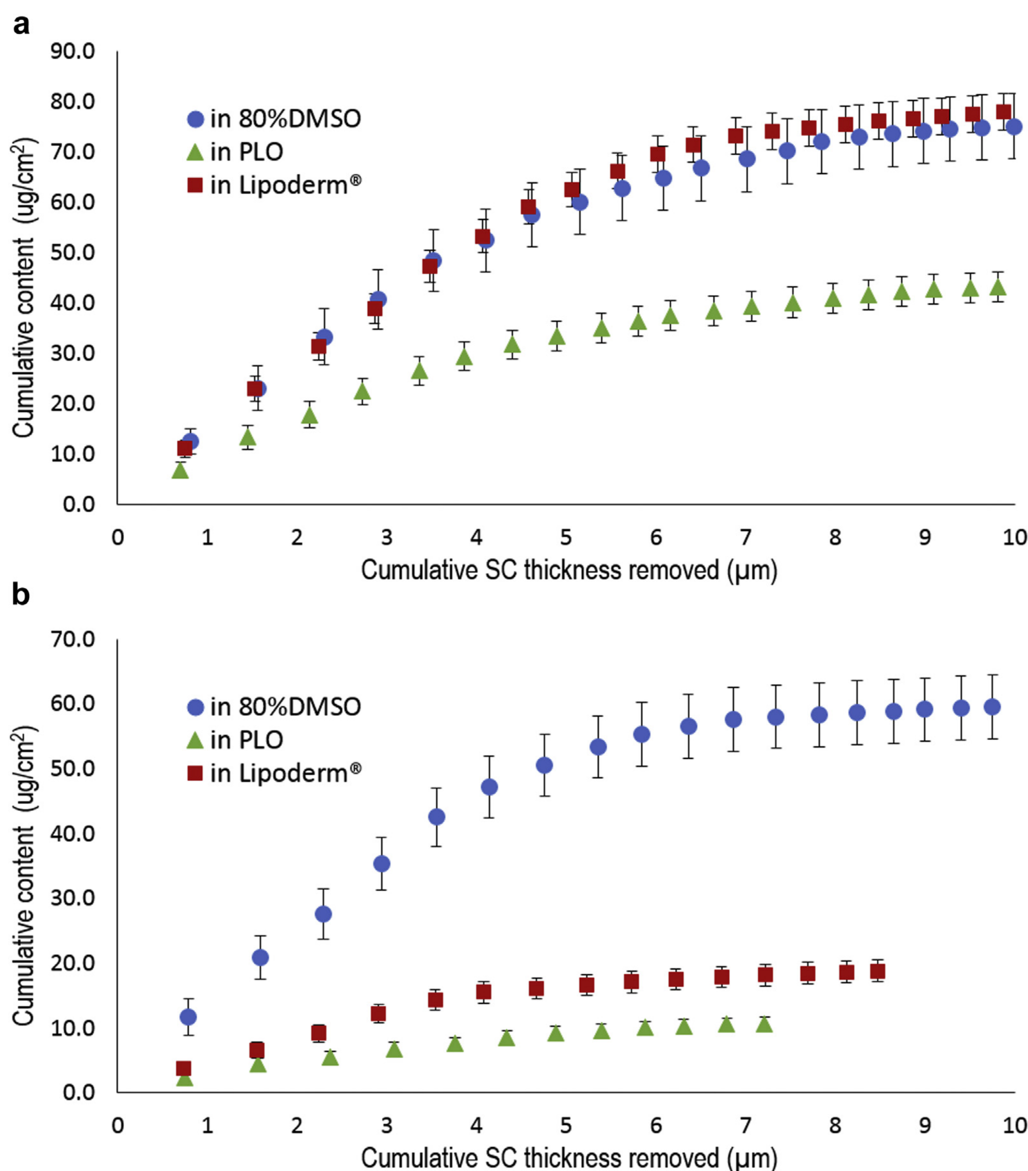

Figure 2. (a) Distribution of fluorescein sodium, and (b) fluorescein (free acid) in stratum corneum (SC) as removed by tape strips. Values are presented as the mean ± SD, $n = 6$.

has an acceptable skin distribution; however, only a relatively small amount could be recovered. In PLO and Lipoderm®, there was no detectable amount after removal of the 12th tape strip from PLO and the 15th tape strip from Lipoderm® (as shown in Fig. 2b).

Figure 3 shows the cumulative amount of probe retained in the skin from different formulations as recovered from tape strips 1-5, 6-10, 11-15, 16-20, and from the stripped skin (viable epidermis + dermis). As indicated in Figure 3a, Lipoderm® cream showed a comparable total skin uptake of fluorescein sodium to 80% DMSO as the vehicle, PLO, however, delivered the least quantity of probe into the skin, especially for fluorescein (free acid) (Fig. 3b). It is interesting to note that, the first 5 tape strips accounted for the majority of the fluorescein sodium absorbed into the stratum corneum with 63.5%, 59.4%, and 60.1% from 80% DMSO, PLO, and Lipoderm®, respectively. Similarly, fluorescein (free acid) recovered from the first 5 tapes accounted for 63.4%, 69.7%, and 74.7% from 80% DMSO, PLO, and Lipoderm®, respectively. Total skin retention of fluorescein sodium from the 3 formulations is in the order of 80% DMSO ≈ Lipoderm® > PLO, and fluorescein (free acid) in the order of 80% DMSO > Lipoderm® > PLO.

Visualization of Probe Distribution in Skin

MPM images were employed to visualize the depth distribution of fluorescence probes in skins treated with fluorescein sodium and fluorescein (free acid) prepared in 80% DMSO, PLO, and Lipoderm® base. The skin surface was defined as the imaging plane of the brightest fluorescence, morphologically characteristic of stratum corneum. Imaging settings were identical for all samples. Setting conditions minimized autofluorescence of the skins, and blank vehicles were confirmed to show no interfering fluorescence.

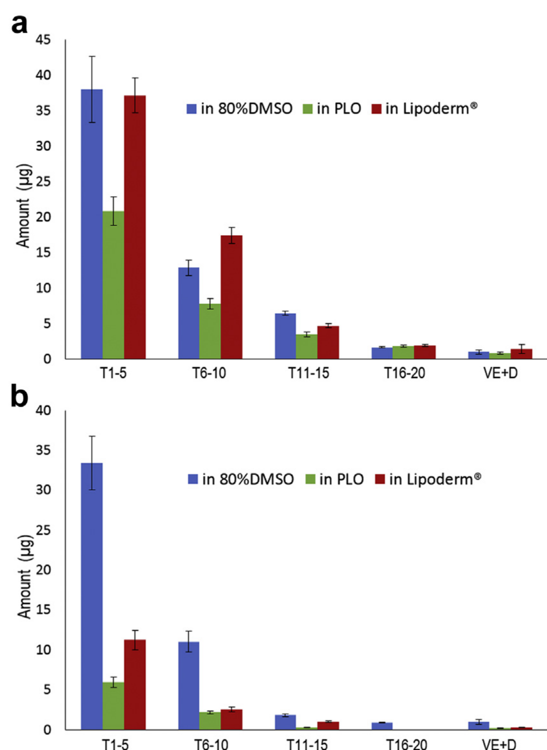

Figure 3. (a) Amounts of fluorescein sodium removed, and (b) amounts of fluorescein (free acid) removed from tape strips 1-5, 6-10, 11-15, 16-20, and retained in the stripped skin (including viable epidermis and dermis [VE + D]) after 28 h permeation study of 3 formulations in porcine ear skin ($n = 6$).

As shown in Figure 4, the observed fluorescence distribution across the skin surface after treatment with fluorescein sodium and fluorescein (free acid) from 80% DMSO was relatively homogenous. However, in most cases, the fluorescence intensity for both probes diminished rapidly with depth when PLO was used as the vehicle (Fig. 5).

Comparing the x-z images of both probes from PLO and Lipoderm® cream bases, overall, Lipoderm® showed brighter fluorescence intensity than PLO base for both probes in the skin. Indeed, the surprisingly high skin penetration of fluorescein sodium from Lipoderm® (Fig. 1a) was supported by the microscopically observed data that fluorescein sodium could penetrate the skin from certain spots on the skin surface, where a brighter fluorescence was detected in the deeper layers of stratum corneum (Fig. 6a1). However, the distribution of fluorescence was not homogenous across the treated skin area; when scanning other parts of the skin, the surface fluoresced weakly, and the intensity was found to disappear rapidly (as shown in Fig. 6a2). In addition, little fluorescence could be detected in the deeper stratum corneum when Lipoderm® base was used (Fig. 6b).

Discussion

In this work, we compared the effects of 2 different transdermal bases on skin permeation, diffusivity, and skin retention of formulated agents. Two fluorescent probes of similar size but different lipophilicity were chosen. It is well known that chemical enhancers are commonly included to improve skin absorption and penetration, where they exert various effects to alter or diminish the barrier function of the stratum corneum. PLO contains lecithin and isopropyl palmitate and is believed to facilitate the diffusion of drug molecules through the stratum corneum by disrupting the lipid layers of the stratum corneum but without damaging them.[4] A number of drugs have been incorporated within PLO for human and animal use as summarized in a previous review.[3] The ingredients of Lipoderm® however are not in the public domain, while the PCCA web site[29] suggests that Lipoderm® is a phospholipid base that contains a proprietary liposomal component which may enhance the skin permeation of a variety of drug actives. Lecithin in PLO also consists primarily of phospholipids. The use of phospholipids or liposomes has been widely investigated, with the suggestion that certain types of phospholipids can diffuse into the stratum corneum to fluidize the highly ordered bilayer structure.[30,31] In this study, we used 80% DMSO as the control vehicle, since DMSO increases skin penetration of drugs by dissolving the stratum corneum lipids.[4]

Lipoderm® base showed better transdermal delivery of fluorescein sodium than 80% DMSO and PLO. It worked similarly when compared to PLO for the delivery of the more lipophilic probe: fluorescein (free acid), where only very minimal amounts crossed the skin. On the other hand, the skin retention of fluorescein (free acid) with Lipoderm® and PLO was also low compared to 80% DMSO.

When correlating the steady-state flux with skin retention, confirmation that each probe's partitioning and thermodynamic activity (as described by skin retention in this study) were highly related to its corresponding skin flux (Fig. 7), which was consistent with previous work.[16,23,32] Skin retention of the probe, as revealed by tape stripping, reflected a reasonable skin penetration through different skin layers, where a certain quantity of the probe is not only retained in superficial layers but also penetrates to a greater depth. Different skin surface cleaning procedures could cause variations in the results, for instance, some formulations were quite viscous and unlikely to be washed off entirely by water, and the residue on the skin would contribute to the total skin retention, though retained only on the surface. We used soapy water to wash

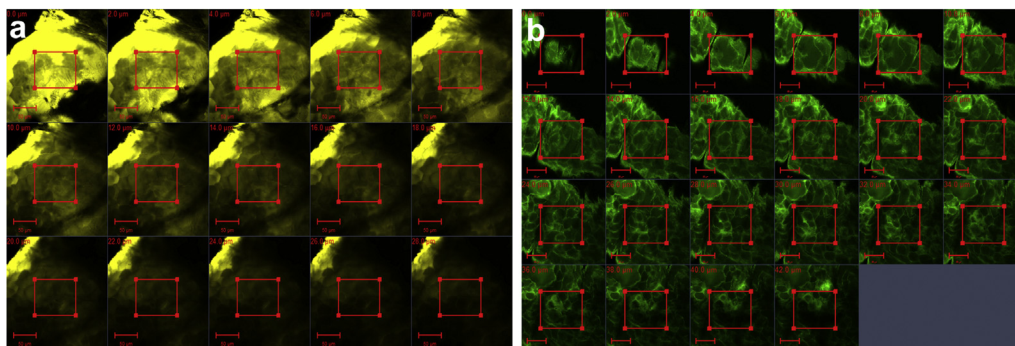

Figure 4. (a) MPM images of porcine skin treated with fluorescein sodium, and (b) fluorescein (free acid) in 80% DMSO, where the serial z stacks of the marked area at different skin depths are presented (the scale bar represents 50 μm).

each skin sample the same way to minimize the superficial residue's influence on the final result. In addition, the use of tape stripping allows monitoring of the probe in deeper layers in contrast to the method of soaking the whole skin disc in an extraction solution. Moreover, tape stripping generates more samples to be processed and analyzed.

It is evident that skin absorption and penetration are highly dependent on the physicochemical properties of the drug active and vehicle or formulation in which it is prepared. As such, it should be noted that the low skin penetration of fluorescein (free acid) may be due to its poor solubility in PLO and Lipoderm® bases, which was 0.12 ± 0.03% and 0.23 ± 0.05%, respectively. Therefore, with a 10% loading, most of the probe incorporated into the cream base remained as a suspension; thus, poor thermodynamic activity would lead to a low skin flux. According to Fick's law, the flux is proportional to the drug concentration in the donor phase, and high solubility usually permits a high drug concentration in the vehicle which can enhance the permeation flux.[33,34]

Another important aspect of the formulation is its pH, and for fluorescein sodium-loaded creams, we found that the pH was increased to 8.0-8.3, and in 80% DMSO, the pH was also increased from 9.2 to 11.3. In contrast, the pH of free acid–loaded creams was only slightly changed and remained in the range of 5.4-6.5, which was close to the pH of the normal skin's surface (i.e., in the range of 5.4-5.9).[35] The pH of the formulation is important and can have a number of effects on the delivery. For example, a disruption of the skin barrier function by alkaline vehicles as reported by other studies[36-38] and the difference of formulation pH could also explain the smaller lag time and higher skin flux of fluorescein sodium when compared to fluorescein (free acid) in both bases. Furthermore, the relative fractions of the free acid and ionized forms in the aqueous phase will vary with pH. It should be noted that the amount of fluorescein free acid is in excess of its intrinsic solubility in these creams, and so the unionized free acid concentration would actually be at its solubility limit. For creams prepared using the fluorescein sodium salt and having relatively high pH, more than 97% of the fluorescein would remain ionized based on a pKa of 6.43.[39] However, in lower pH formulations prepared using the fluorescein free acid, 85.5% of dissolved fluorescein free acid becomes ionized in 80% DMSO, 37.1% becomes ionized in the aqueous phase of PLO, and only 10.5% was ionized in the aqueous phase of Lipoderm® cream. Given the low solubility of the free acid in the cream bases and the reduced fraction of the ionized form at low pH, ultimate concentrations of the ionized form would be 0.04% and 0.02% in the free acid-loaded PLO and Lipoderm® creams, respectively, which was far below the concentration of ions in the sodium salt–loaded PLO and Lipoderm® creams. This higher concentration of the ions in the sodium salt–loaded cream bases explains its higher thermodynamic activity.

In addition, the physiological pH environment of the skin could change the form of fluorescein acid, and when the free acid penetrates into the dermis facing the receptor medium, the majority of fluorescein should be in its conjugate base form under that environment. Unfortunately, the current setting for microscopy experiments was not able to differentiate which form has been detected. From the estimated fractions and concentration values, there were no substantial ions formed in these 2 creams. Our data also showed that fluorescein free acid had a poor skin penetration compared to its sodium salt; thus, we believe that the ionized form would most likely permeate through the skin. This is in agreement with the study of a series of nonsteroidal anti-inflammatory agents, where most of the drugs were ionized in the donor.[17] In addition, several published studies have shown evidence that for some drug candidates, salt formation could improve permeability through the skin, including biphenylacetic acid,[40] diclofenac,[41] and buprenorphine.[42] Therefore, we think that a suitable salt former with desirable

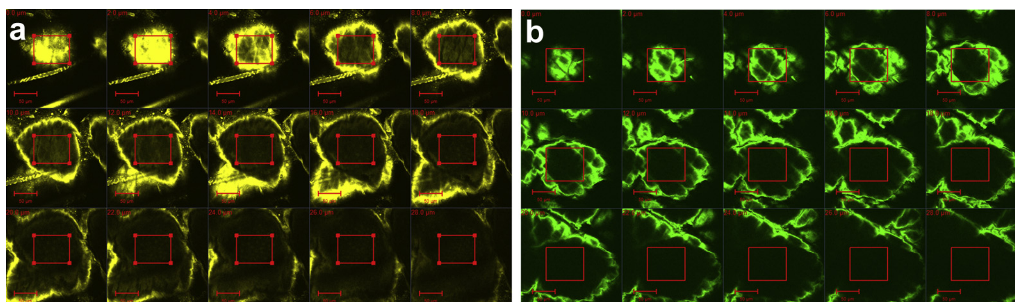

Figure 5. (a) MPM images of porcine skin treated with fluorescein sodium, and (b) fluorescein (free acid) in PLO, where the serial z stacks of the marked area at different skin depths are presented (the scale bar represents 50 μm).

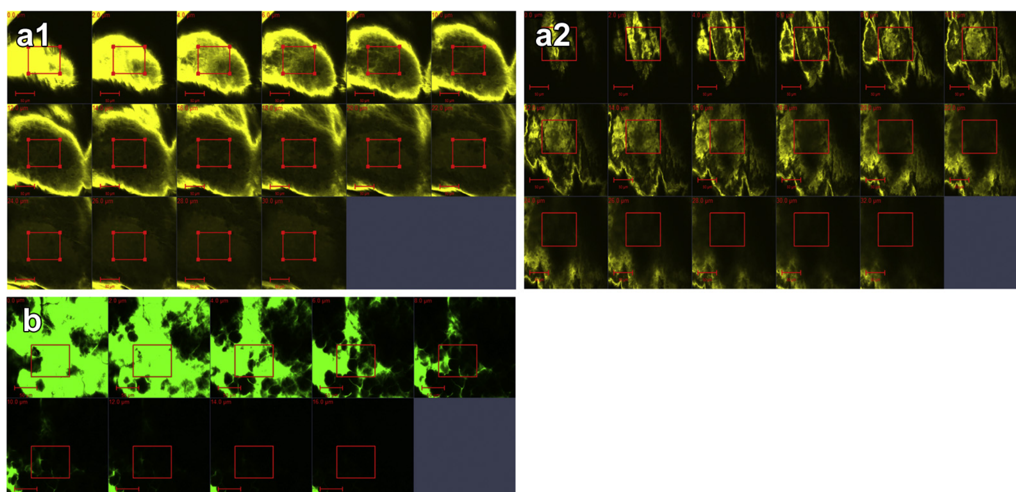

Figure 6. (a) MPM images of porcine skin treated with fluorescein sodium (1 and 2 represent images of different sections of the skin), and (b) fluorescein (free acid) in Lipoderm®, where the serial z stacks of the marked area at different skin depths are presented (the scale bar represents 50 μm).

solubility properties[41] was one of the main factors for an enhanced skin penetration.

Besides tape stripping, MPM was also undertaken to obtain the depth distribution of probes in the skin, mainly in the stratum corneum. Imaging data for fluorescein (free acid) in these vehicles were consistent with the results from the skin penetration study, where more molecules from 80% DMSO diffused through the skin with time, leading to much higher fluorescence intensity observable deeper into the skin (Fig. 4b). In contrast, only minimal fluorescein (free acid) penetrated when PLO and Lipoderm® bases were used; indeed, not much fluorescence was detected in the skin except within a bright surface layer (Figs. 5b and 6b). We also observed uneven distribution of probes across the skin surface and deeper layers in the x-y plane when these 2 cream bases were used. It seems that the affinity between the skin surface and the bases may affect skin absorption and penetration. While a fluidized vehicle, for example, 80% DMSO, showed a much more homogenous distribution of probes in the x-y plane. Imaging data were relatively comparable between skin samples with different treatments. We kept settings the same while performing z stacks for all skin samples, as high laser power could easily photo bleach the surface where most of the probe remained. We also found some "hot spots" in skin samples treated by fluorescein sodium in Lipoderm® with some fluorescence observed deep into the skin. This may be due to some defects in the skin surface; however, all these skin samples passed the integrity testing while setting up the Franz cell diffusion tests. Overall, fluorescence intensity was not evenly distributed across the treated skin area.

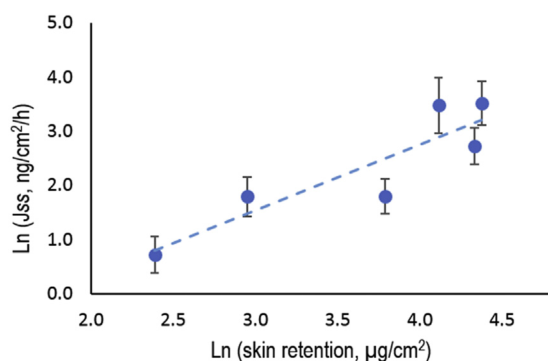

Figure 7. Linear relationship between ln (*Jss*) and ln (skin retention), $r^2 = 0.80$.

A suitable transdermal base features good stability, low skin irritancy, compatibility with drug actives, and most importantly, ability to transport the active deep into the skin. The skin is a highly attractive application site for directed drug delivery, but delivery through skin is still very challenging. The excipients incorporated into the transdermal base need to be carefully selected, with concentration also playing an important role. PLO consists of lecithin and Pluronic F127; one study showed that increasing lecithin, decreases the cumulative amount of flurbiprofen released from the base. This may be due to the high viscosity produced by the extensive formation of a network-like structure.[43] A better understanding of drug-vehicle-skin interaction is quite useful in formulating such bases. Several studies have advocated that solubility parameters should be considered as numerical indicators to show the extent of interaction.[44-47] Studies by Dias et al.[48,49] showed that decanol and octanol which have 2D solubility parameters close to that of the stratum corneum, enhanced the flux of 3 selected permeants; however, there was no simple correlation between flux across epidermis and the values previously reported for silicone. Abbott[50] proposed to use 3D Hansen solubility parameters, which characterize the dispersion, polarity, and hydrogen-bonding components to cover any polar or hydrogen-bonding interactions between drug-vehicle-skin. However, this hypothesis remains to be examined when applied to a complex base which contains many and various excipients. Our results suggest that solubility characteristics of the probe: effective concentration in the transdermal base and in the skin as expressed by skin retention and the pH of the probe-loaded formulation should be well characterized to achieve a successful transdermal preparation.

Conclusion

This work systematically examined the effects of PLO and Lipoderm® on skin permeation, diffusivity, and skin retention of 2 probes. This is the first investigation of transdermal delivery of 2 similar-sized probes: the sodium salt form and free acid of fluorescein as prepared in 2 popular transdermal bases. This work suggests that the use of different transdermal bases can lead to significantly different skin penetration, absorption, and retention of the formulated agents with various lipophilicities. These results show that with the same concentration of fluorescein sodium in

these 2 bases, Lipoderm® leads to greater skin penetration and absorption of fluorescein sodium compared to PLO base, while there was no significant difference in transdermal delivery of fluorescein (free acid), which was more lipophilic and had poor solubility when incorporated into either base. The solubility of the active pharmaceutical ingredient in the base and in the skin would be key factors governing its percutaneous absorption.

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References

1. Nornoo AO, Wulz J, Yoon H, Nan Y, Lese M. Impact of the chemical and physical stability of ketoprofen compounded in various pharmaceutical bases on its topical and transdermal delivery. *Pharm Dev Technol*. 2016;21(2):204-213.
2. Willimann H, Walde P, Luisi PL, Gazzaniga A, Stroppolo F. Lecithin organogel as matrix for transdermal transport of drugs. *J Pharm Sci*. 1992;81(9):871-874.
3. Almeida H, Amaral MH, Lobao P, Lobo JM. Pluronic(R) F-127 and Pluronic Lecithin Organogel (PLO): main features and their applications in topical and transdermal administration of drugs. *J Pharm Pharm Sci*. 2012;15(4):592-605.
4. Loyd AV. The history of pluronic lecithin organogel: an interview with Marty Jones, BSPharm, FACA, FIACP. *Int J Pharm Compd*. 2003;7(3):181-183.
5. Drummond ES, Maker G, Birklein F, Finch PM, Drummond PD. Topical prazosin attenuates sensitivity to tactile stimuli in patients with complex regional pain syndrome. *Eur J Pain*. 2016;20(6):926-935.
6. Heustess A, Spigener S, Sweitzer S, Romero-Sandoval A, Asbill S. Analgesic efficacy and transdermal penetration of topical gabapentin creams: finding an optimal dose and pre-treatment time. *Int J Pharm Compd*. 2015;19(2):167-173.
7. Bassani AS, Banov D, Lehman PA. Evaluation of the percutaneous absorption of promethazine hydrochloride, in vitro, using the human ex vivo skin model. *Int J Pharm Compd*. 2008;12(3):270-273.
8. Bassani AS, Banov D, Phan H. Characterization of the percutaneous absorption of ketoprofen using the Franz skin finite dose model. *Postgrad Med*. 2016;128(2):262-267.
9. Bassani AS, Banov D. Evaluation of the percutaneous absorption of ketamine HCl, gabapentin, clonidine HCl, and baclofen, in compounded transdermal pain formulations, using the Franz finite dose model. *Pain Med*. 2016;17(2):230-238.
10. Karande P, Mitragotri S. Enhancement of transdermal drug delivery via synergistic action of chemicals. *Biochim Biophys Acta*. 2009;1788(11):2362-2373.
11. Dragicevic N, Atkinson JP, Maibach HI. Chemical penetration enhancers: classification and mode of action. In: *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement*. Berlin Heidelberg: Springer-Verlag; 2015:11-27.
12. Barry BW. Lipid-protein-partitioning theory of skin penetration enhancement. *J Control Release*. 1991;15(3):237-248.
13. Williams AC, Barry BW. Penetration enhancers. *Adv Drug Deliv Rev*. 2004;56(5):603-618.
14. van Kuijk-Meuwissen ME, Mougin L, Junginger HE, Bouwstra JA. Application of vesicles to rat skin in vivo: a confocal laser scanning microscopy study. *J Control Release*. 1998;56(1-3):189-196.
15. Alvarez-Roman R, Naik A, Kalia YN, Guy RH, Fessi H. Enhancement of topical delivery from biodegradable nanoparticles. *Pharm Res*. 2004;21(10):1818-1825.
16. Zhang Q, Li P, Roberts MS. Maximum transepidermal flux for similar size phenolic compounds is enhanced by solvent uptake into the skin. *J Control Release*. 2011;154(1):50-57.
17. Cordero JA, Alarcon L, Escribano E, Obach R, Domenech J. A comparative study of the transdermal penetration of a series of nonsteroidal antiinflammatory drugs. *J Pharm Sci*. 1997;86(4):503-508.
18. Hadgraft J, Valenta C. pH, pK(a) and dermal delivery. *Int J Pharm*. 2000;200(2):243-247.
19. Mahrhauser DS, Reznicek G, Gehrig S, et al. Simultaneous determination of active component and vehicle penetration from F-DPPC liposomes into porcine skin layers. *Eur J Pharm Biopharm*. 2015;97(Pt A):90-95.
20. Subongkot T, Ngawhirunpat T. Effect of liposomal fluidity on skin permeation of sodium fluorescein entrapped in liposomes. *Int J Nanomedicine*. 2015;10:4581-4592.
21. Monteiro-Riviere N, Riviere JE. The pig as a model for cutaneous pharmacology and toxicology research. In: Tumbleson ME, Shook LB, eds. *Advances in Swine in Biomedical Research*. New York: Plenum Press; 1996:425-458.
22. Jacobi U, Kaiser M, Toll R, et al. Porcine ear skin: an in vitro model for human skin. *Skin Res Technol*. 2007;13(1):19-24.
23. Zhang Q, Grice JE, Li P, Jepps OG, Wang GJ, Roberts MS. Skin solubility determines maximum transepidermal flux for similar size molecules. *Pharm Res*. 2009;26(8):1974-1985.
24. Organisation for Economic Co-operation and Development Guidance Document for the Conduct of the Skin Absorption Studies. OECD Series on Testing and Assessment No. 28. 2004.
25. Organisation for Economic Co-operation and Development OECD Guideline for the Testing of Chemicals. Skin Absorption: In Vitro Method. Guideline 428. 2004.
26. Klang V, Schwarz JC, Hartl A, Valenta C. Facilitating in vitro tape stripping: application of infrared densitometry for quantification of porcine stratum corneum proteins. *Skin Pharmacol Physiol*. 2011;24(5):256-268.
27. Anderson RL, Cassidy JM. Variation in physical dimensions and chemical composition of human stratum corneum. *J Invest Dermatol*. 1973;61(1):30-32.
28. Haque T, Crowther JM, Lane ME, Moore DJ. Chemical ultraviolet absorbers topically applied in a skin barrier mimetic formulation remain in the outer stratum corneum of porcine skin. *Int J Pharm*. 2016;510(1):250-254.
29. Bugden DL. Identification and antibiotic susceptibility of bacterial isolates from dogs with otitis externa in Australia. *Aust Vet J*. 2013;91(1-2):43-46.
30. Kirjavainen M, Monkkonen J, Saukkosaari M, Valjakka-Koskela R, Kiesvaara J, Urtti A. Phospholipids affect stratum corneum lipid bilayer fluidity and drug partitioning into the bilayers. *J Control Release*. 1999;58(2):207-214.
31. Vanic Z. Phospholipid vesicles for enhanced drug delivery in dermatology. *J Drug Discov Develop Deliv*. 2015;2(1):1010.
32. Zhang Q, Li P, Liu D, Roberts MS. Effect of vehicles on the maximum transepidermal flux of similar size phenolic compounds. *Pharm Res*. 2013;30(1):32-40.
33. Guy RH. Current status and future prospects of transdermal drug delivery. *Pharm Res*. 1996;13(12):1765-1769.
34. Ceschel G, Bergamante V, Maffei P, et al. Solubility and transdermal permeation properties of a dehydroepiandrosterone cyclodextrin complex from hydrophilic and lipophilic vehicles. *Drug Deliv*. 2005;12(5):275-280.
35. Schmid-Wendtner MH, Korting HC. The pH of the skin surface and its impact on the barrier function. *Skin Pharmacol Physiol*. 2006;19(6):296-302.
36. Antoine JL, Contreras JL, Van Neste DJ. pH influence of surfactant-induced skin irritation. A non-invasive, multiparametric study with sodium laurylsulfate. *Derm Beruf Umwelt*. 1989;37(3):96-100.
37. Ananthapadmanabhan KP, Lips A, Vincent C, et al. pH-induced alterations in stratum corneum properties. *Int J Cosmet Sci*. 2003;25(3):103-112.
38. Paudel KS, Milewski M, Swadley CL, Brogden NK, Ghosh P, Stinchcomb AL. Challenges and opportunities in dermal/transdermal delivery. *Ther Deliv*. 2010;1(1):109-131.
39. Sjöback R, Nygren J, Kubista M. Characterization of fluorescein-oligonucleotide conjugates and measurement of local electrostatic potential. *Biopolymers*. 1998;46(7):445-453.
40. Pawar V, Naik P, Giridhar R, Yadav MR. Enhancing skin permeation of biphenylacetic acid (BPA) using salt formation with organic and alkali metal bases. *Sci Pharm*. 2015;83(1):191-205.
41. Stahl PH, Sutter B. Salt selection. In: Hilfiker R, ed. *Polymorphism: In the Pharmaceutical Industry*. Weinheim, Germany: Wiley-VCH; 2006:309-332.
42. Roy SD, Roos E, Sharma K. Transdermal delivery of buprenorphine through cadaver skin. *J Pharm Sci*. 1994;83(2):126-130.
43. Pandey MS, Belgamwar VS, Surana SJ. Topical delivery of flurbiprofen from pluronic lecithin organogel. *Indian J Pharm Sci*. 2009;71(1):87-90.
44. Sloan KB, Koch SA, Siver KG, Flowers FP. Use of solubility parameters of drug and vehicle to predict flux through skin. *J Invest Dermatol*. 1986;87(2):244-252.
45. Groning R, Braun FJ. Three-dimensional solubility parameters and their use in characterising the permeation of drugs through the skin. *Pharmazie*. 1996;51(5):337-341.
46. Hashiguchi T, Yasutake T, Manako T, Otagiri M. In vitro percutaneous absorption of prednisolone derivatives based on solubility parameter. *Int J Pharm*. 1997;158(1):11-18.
47. Cross SE, Pugh WJ, Hadgraft J, Roberts MS. Probing the effect of vehicles on topical delivery: understanding the basic relationship between solvent and solute penetration using silicone membranes. *Pharm Res*. 2001;18(7):999-1005.
48. Dias M, Hadgraft J, Lane ME. Influence of membrane-solvent-solute interactions on solute permeation in model membranes. *Int J Pharm*. 2007;336(1):108-114.
49. Dias M, Hadgraft J, Lane ME. Influence of membrane-solvent-solute interactions on solute permeation in skin. *Int J Pharm*. 2007;340(1-2):65-70.
50. Abbott S. An integrated approach to optimizing skin delivery of cosmetic and pharmaceutical actives. *Int J Cosmet Sci*. 2012;34(3):217-222.