Evaluation of three base creams effects on skin penetration & retention of fluorescein free acid

A study conducted by:

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University of Victoria

Abstract

This study was designed to evaluate the effects of three base creams, **TD Lipo**, typical liposomal base, and typical PLO-based gel effects on the permeation and retention of a probe (fluorescein-free acid, FFA) through the skin. These two parameters were evaluated by using porcine skin, as a standard model instead of human skin. Penetration test was done by measuring the amount of FFA passed through the skin by using a Franz cell device and tape stripping was used to get the retention and depth distribution profiles in the skin. In addition, fluorescent microscopy images were taken to confirm the results. The results showed that TD Lipo enhanced both penetration and retention of FFA compared to other base formulations. Microscopic images confirmed the higher retention content of FFA and its concentration gradient from the source of treatment. This work showed that TD Lipo exerted higher penetration and retention capacities for FFA.

Literature Review and Aim of Study

It is believed that **TD Lipo**, with its qualities for efficient transdermal delivery systemof drugs, must exert greater results in comparison to conventional bases (typical liposomal base and typical PLO-based gel). The aim of this study is to evaluate the **potency** of TD Lipo in terms of drug **penetration and retention**, and this is the first study in this field.

Introduction

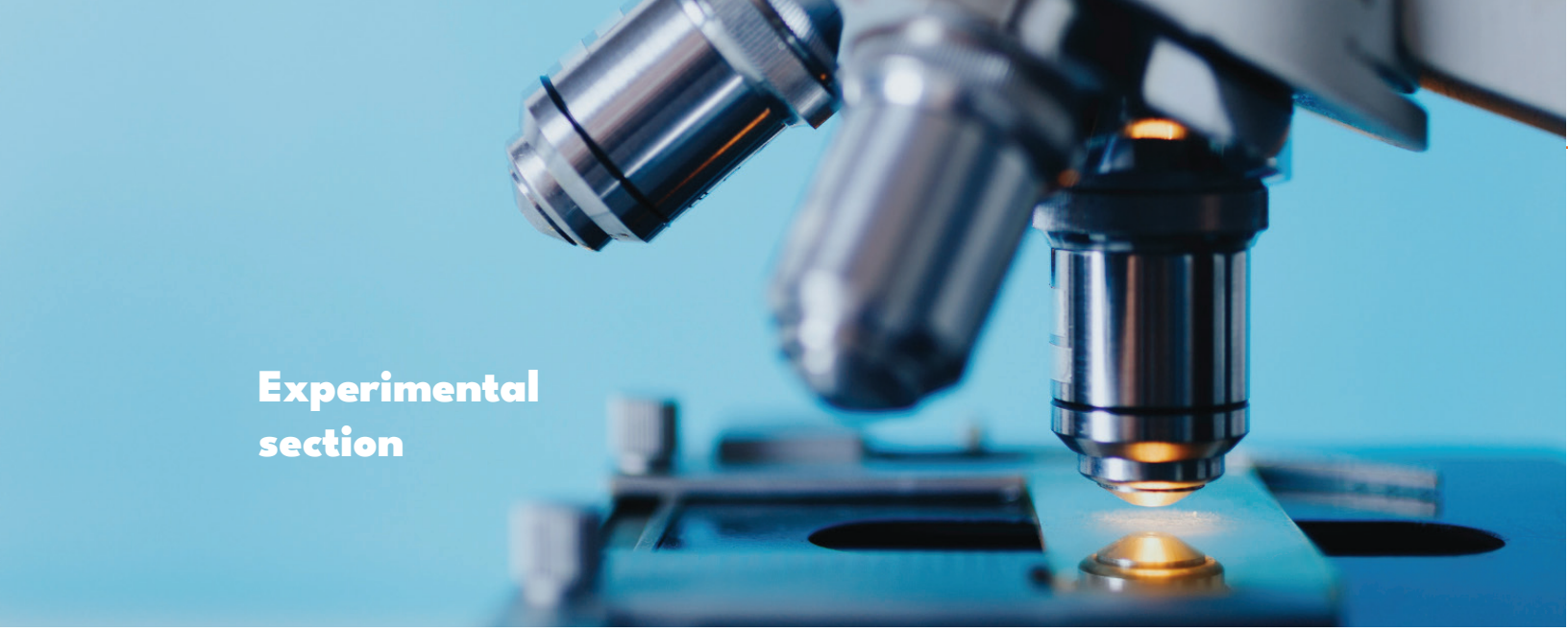
Nowadays, active ingredient delivery via the skin has been an intriguing and demanding field of pharmaceutical research, as this route of administration provides us with numerous benefits. Transdermal bases should be compatible with a wide range of therapeutic agents and actively transport medications through the skin to the site of action. One of the novel strategies to enhance drug delivery through the skin is the destruction of the intact stratum corneum (SC), the main barrier against drug entry into and via the skin. To remove this challenge, significant efforts have been made to improve percutaneous drug absorption, including the addition of chemical penetration promoters into the skin.[1-5]

Conventionally, Pluronic lecithin organogel (typical PLO-based gel), one of the most well- known traditional transdermal drug delivery systems, and typical liposomal base, the newer one, has been widely used in this area due to their product stability and in vivo skin absorbance.[6,7]

TD Lipo, a liposome-based transdermal drug delivery system, is able to pass active lipophilic pharmaceutical ingredients into and through the skin. TD Lipo is designed in a way to provide more transdermal delivery of lipophilic drugs such as diclofenac, progesterone, and testosterone. More than enhancing the drug penetration ability, it offers a cosmetically appealing texture, a non-greasy cream base, and enhanced dermal penetration of lipophilic active pharmaceutical ingredients (APIs). In addition, TD Lipo is able to incorporate APIs and integrate a higher percentage of APIs than traditional PLO-based gel.[8]

TD Lipo is a perfect option for topical pharmaceuticals, skincare products, and cosmetics because it has been made to improve the absorption of active chemicals into the skin. Creams, lotions, ointments, and gels are just a few of the many applications of this product. Because of its exceptional emollient qualities and distinctive formulation, it is a fantastic choice for dry and sensitive skin.[8]

In this study, we tried to evaluate the penetration and retention capacity of fluorescein (free acid, FFA) as a probe through porcine skin by using typical PLO-based gel, typical liposomal base, and TD Lipo. In addition, depth distribution in the skin and gradient concentration of the probe was then visualized by using fluorescent microscopy.



Experimental section

Materials

Fluorescein (free acid, >95%)
phosphate-buffered saline
bovine serum albumin
propylene glycol
dimethyl sulfoxide (DMSO)
Typical liposomal base cream
typical PLO-based gel base cream
methanol

Analytical Method

FFA concentrations were determined by using fluorescence spectroscopy. Three replicates of 100 µL for each sample were pipetted into a black 96-well plate, and fluorescence was measured in a microplate reader (Tecan Infinite M Nano) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm.[1,9,10]

Formulation Preparation

The typical PLO-based gel with probe was prepared by loading FFA 10% to a blank PLO base. In the case of typical liposomal base cream, FFA was added to the typical liposomal base along with 10% propylene glycol as a wetting agent. All formulations were kept at room temperature before application and used within 2 weeks. 80% DMSO was used as a positive control vehicle in this study. TD Lipo loaded with the probe was prepared by triturating 0.5g FFA to form a uniformly fine powder mixture. Then 0.25 mL of isopropyl alcohol was added to the mixture to levigate, followed by 10.125 mL of propylene glycol to form a smooth paste. Finally, 5 g of TD Lipo base cream was added to the paste and mixed well.[1]

In Vitro Skin Permeation Study

It has become commonplace to use porcine ear skin in place of human skin. Large white pigs' ears were employed as an ex-situ skin model. Excess hair and fatty tissue were carefully removed to provide full thickness skin (800±50 µm). The skin was kept at -20°C and slowly defrosted at 4°C before being cleaned with water. According to a prior study, electrical resistance was used to determine the integrity of the skin.[1,11-13]

With a diffusion area of 1cm2, the Franz cell (PermeGear, Hellertown, PA) clamped the skin sample while the receptor chamber was filled with 5 mL of 4% bovine serum albumin in PBS, Figure 1. A water jacket maintained the temperature of the receptor fluid at 32°C, while a magnetic stirring bar continuously mixed it. A glass rod was used to spread each formulation equally across the diffusion region after being applied to the skin's surface in quantities of 100 mg. The Parafilm tape was used to seal the donor chamber.[1,14,15]

At 2, 4, 6, 8, 12, 24, and 28 hours, a 500 mL sample was taken through the sampling port and replaced with brand-new receptor media. Within one month of sampling, samples were either frozen at 20 °C for later high-performance liquid chromatography analysis or kept in a refrigerator and analyzed within two days. Frozen samples were thawed to room temperature on the day of the analysis, and 100 mL of the sample was pipetted into a dark 96-well plate for testing. For samples where the concentration of the probe was discernible but not measurable, 300 mL was obtained and combined with 100 mL of methanol. A supernatant sample of 350 µL was collected, dried with nitrogen gas at 45 degrees, and then dissolved in 100 mL of methanol.

Skin Retention

The diffusion cells were disassembled after the last time point's collection of the receptor fluid, and any extra formulation on the skin was removed with moist tissue. The skin was washed with two cotton swabs and a mild liquid soap solution, rinsed for five seconds under running water, and then dried with a tissue.

By consecutively tape stripping 12 times with D-Squame® sampling discs (CuDerm Co.), as previously reported, the SC at the treatment location was removed.[1,16] The peeled skin and the discs were each put into a vial, and fluorescein probes were extracted using 0.6 mL of 80% methanol while being gently stirred for 24 hours. Then the amount of FFA was assessed by a microplate reader. The removed blank tapes' methanolic solutions served as the tape- stripping samples' controls.

Depth Distribution by Fluorescent Microscopy

Similar to in vitro Franz cell penetration tests, skin samples were produced for microscope imaging. Only the SC side of the blank vehicle was used to create the negative control samples. Each skin sample was cleansed on the surface and then placed on a microscope slide with 20 mL saline on the SC surface, covered with a coverslip. 50 µm thick skin slices were imaged with a Zeiss Axio Observer 5 fluorescent microscope (Zeiss, Germany). The samples were excited at 800 nm, and fluorescence signals were recorded from 450 to 620 nm. Z-stack images were obtained at the end of the test.

Results

Skin Penetration Studies

The average cumulative amount of FFA penetration through the skin over time is shown in Figure 2. As it is shown, the skin penetration of FFA was greatly enhanced by the TD Lipo base compared to typical PLO-based gel and typical liposomal base bases, but relatively lower than 80% DMSO as a positive vehicle. There was a minimum amount of probe concentration in the receptor medium at the base of a typical PLO-based gel. The average total amount of FFA that pierced the skin was significant at all sampling times when we compared TD Lipo with a typical PLO-based gel base. Though TD Lipo showed a higher amount of FFA skin penetration compared to typical liposomal base, the differences were all significant except in the times of the first and last sampling times (2 and 28 h, respectively). The amount of difference between 80% DMSO and TD Lipo was greatly increased as sampling times went by, and this was significant (Table 1).

Figure 1. Skin permeation profiles of FFA from different formulations

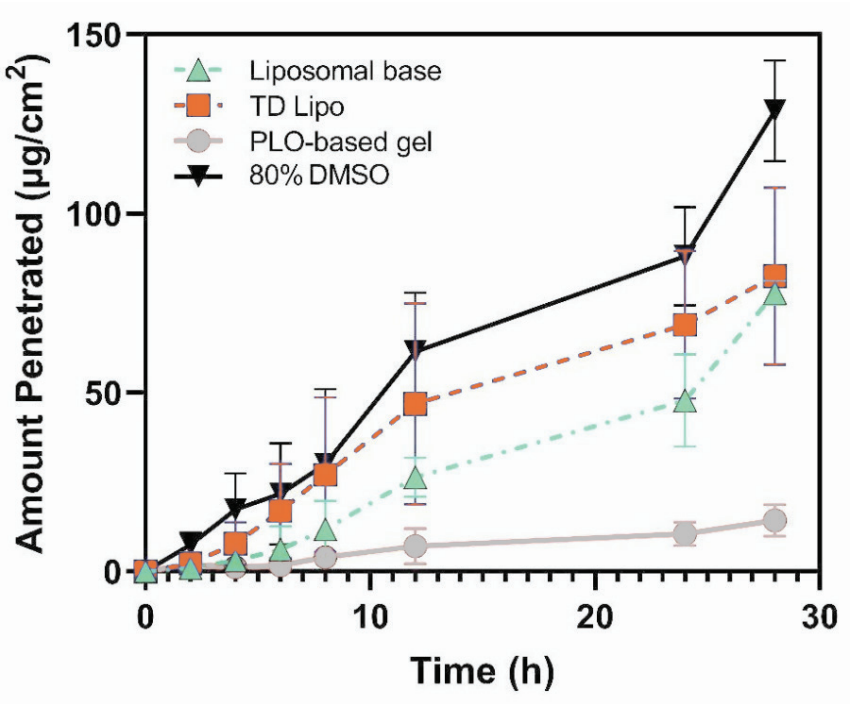
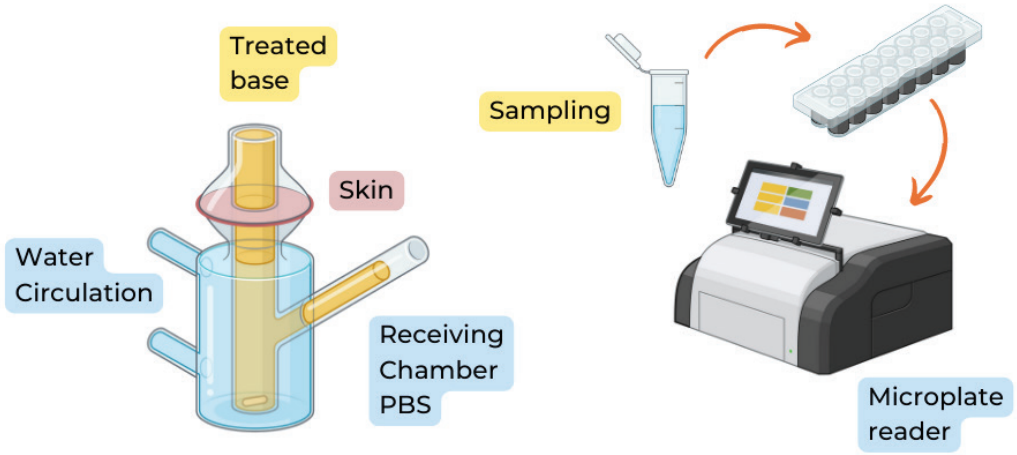


Table 1. Average amount of FFA penetrated in the skin (µg/cm2)

Time	TD Lipo	PLO-Based Gel	80% DMSO	Other Liposomal Base
2	2.24	2.01	7.44	0.99
4	7.8	1.19	17.37	3.11
6	16.89	2.28	21.72	6.13
8	27.09	4.02	30.03	11.78
12	46.79	7.1	61.43	26.31
24	68.95	10.48	88.06	47.78
28	82.57	14.24	128.7	77.8

Figure 2. Schematic presentation of Franz cell and its process for penetration test



Skin Retention

Following the application of the various formulations, average FFA amounts were recovered from each tape strip collected at the end of the skin penetration testing. Altogether, as the number of tape strips and the depth into the SC increased, the recovered amount of both probes from each formulation gradually decreased.

Figure 3 shows the amount of probe retained in the strip after each tape strip from different formulations (the test was repeated twice). As it is evident, the amount in 80% DMSO showed a comparable total skin uptake of FFA to other formulations. It is interesting to note that TD Lipo tape strips accounted for the majority of the FFA absorbed into the SC compared to other formulations, and this difference was significant in many spots, as shown in Table 2.

Figure 3. Amount of removed FFA after each tape strip

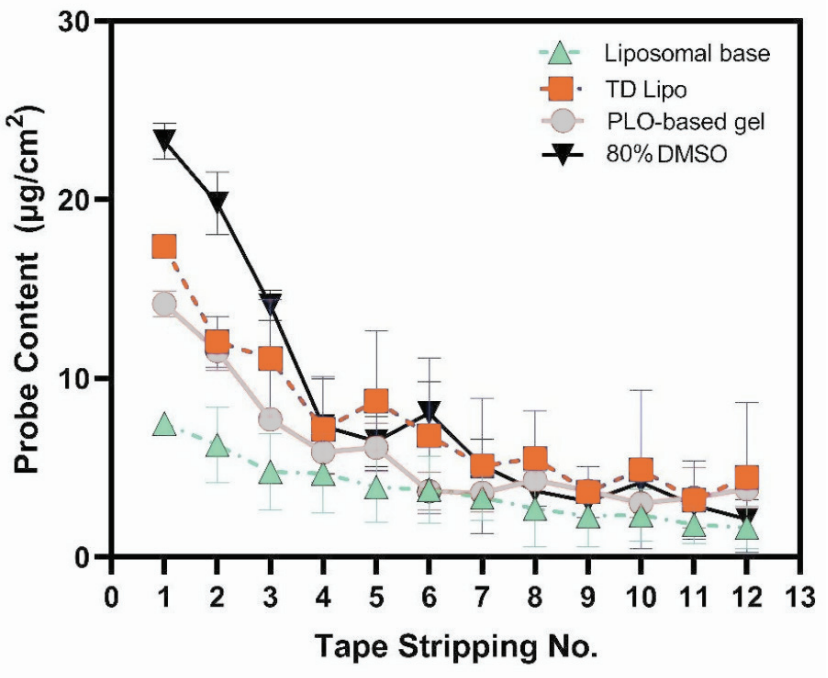
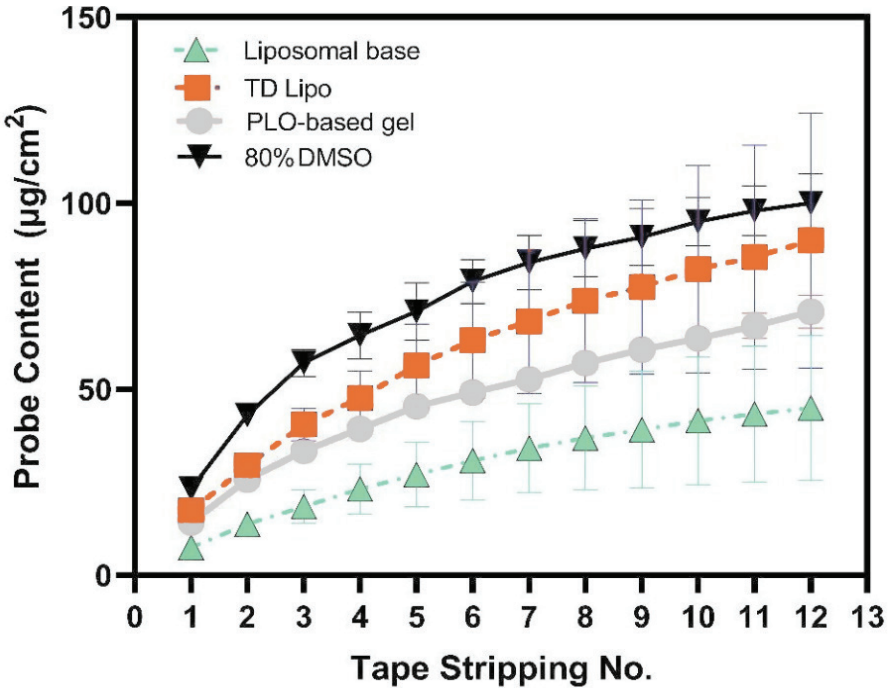


Table 2. Average amount of FFA retained in the skin (µg/cm2)

Tape Strip	TD Lipo	Liposomal Base	PLO-based Gel	80% DMSO
1	17.37	7.47	14.17	23.26
2	29.41	13.73	25.7	43.04
3	40.52	18.5	33.41	57.11
4	47.66	23.17	39.29	64.43
5	56.4	27.08	45.42	70.91
6	63.2	30.83	49.1	78.95
7	68.29	34.16	52.68	84.08
8	73.82	54.37	56.99	87.77
9	77.47	39.15	60.69	90.88
10	82.36	41.5	63.69	95.05
11	85.55	43.31	67	97.95
12	90	44.94	70.8	100.05

Figure 4. Cumulative FFA content after tape stripping



The cumulative retention profile of FFA is shown in Figure 4. The cumulative amount of FFA retained in the skin was significantly higher in TD Lipo in comparison with typical liposomal bases and typical PLO-based gel bases. This significant difference was seen at all times of sampling between TD Lipo and typical liposomal base, and similarly, this difference was significant between TD Lipo and typical PLO-based gel after 5 tape strips and continued until the end of the test. Similarly, 80% DMSO as the vehicle showed a significant total skin uptake of FFA compared to all base formulations.

Visualization of Probe Distribution in Skin

The depth distribution of FFA images was visualized by using a fluorescent microscope in the skin treated with different base formulations. Cross-sectional slices were prepared at the end of the test, and images were taken through each formulation. Imaging settings were identical for all samples.

As demonstrated in Figure 5, the fluorescence pattern of the skin surface after treatment with FFA from TD Lipo was relatively lower than other formulation bases. It was a sign of its capacity to induce higher retention compared to the others. However, the distribution pattern of the drug based on its fragrance intensity showed that probe retention in TD Lipo was higher and more predictable than in other formulations, as we could detect some spots with a non-uniform distribution area.

Comparing the images of three bases showed brighter fluorescence intensity and a less homogenous pattern in both typical liposomal base and typical PLO-based gel than TD Lipo bases. These data confirmed the retention and penetration results that TD Lipo has a higher potency for overall retention and passing the probe through itself. Also, the concentration gradient appeared through the SC as the intensity of fluorescence was lower at the side of the treated area, and this gradient helps the probe penetrate deeper into the skin.

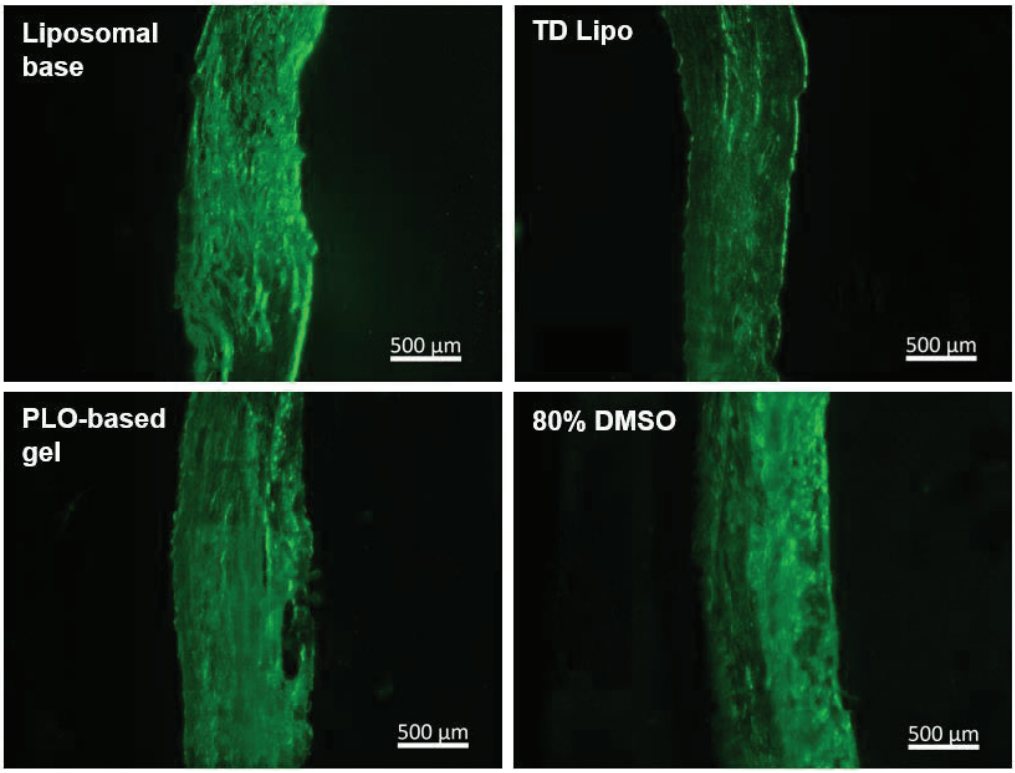


Figure 5. Microscopic images of FFA retention after completing the test

Discussion

Using various transdermal bases can result in noticeably varied skin penetration, absorption, and retention of a probe. In this study, we investigated the effects of three distinct transdermal bases on the skin penetration, diffusivity, and retention of an FFA and evaluated the specific effects of TD Lipo on these two features.

To evaluate our new base, TD Lipo, we decided to compare its retention and penetration capacity with two conventional bases, a liposomal base, and a PLO-based gel, using FFA as a probe and 80% DMSO as the positive control vehicle. typical PLO-based gel contains lecithin and isopropyl palmitate and is able to facilitate the diffusion of drug molecules through the stratum corneum. A liposomal base is a phospholipid base that contains a proprietary liposomal component that may enhance the skin permeation of a variety of drug actives.

Our results showed that TD Lipo has better transdermal delivery of FFA than typical liposomal base and typical PLO-based gel, but lower than 80% DMSO. These results were aligned with the retention test results, which showed that TD Lipo was more potent than the other two bases in terms of retaining the probe. In other words, TD Lipo was able to not only penetrate the higher amount of probe through the skin but also retain an excessive quantity of probe in the skin. Consequently, penetration and retention as two vital items of skin drug delivery were stronger in TD Lipo than in other bases.

By removing the tape, it was possible to see how much of the probe was maintained in the skin. Additionally, using tape stripping enables monitoring of the probe in deeper layers. Tape stripping showed us the distribution of the probe into the skin, and for TD Lipo, it was significantly higher than other bases. This difference was significant between TD Lipo and typical PLO-based gel at all sampling times and can be considered proof of depth penetration into the SC layer. Similarly, the cumulative probe contents were significantly higher in TD Lipo than in typical PLO-based gel. The data from the penetration study were consistent with the results from the skin retention study, where more molecules from TD Lipo diffused through the skin with time, leading to a much higher concentration in diffusion cells.

A comparison between TD Lipo and the typical liposomal base showed that the amount of FFA retained in the SC was significantly higher in TD Lipo than in the typical liposomal base, and it was in parallel with the amount of FFA penetrated through the SC. One of the most important factors in passing the probe through the SC is the concentration shift through the layer. As it was shown in MMP images, the fluorescence intensity between the sides of the source of FFA was gradually increased to the other side (receiving side), which was a reason for the penetration of the higher amount of FFA through the SC. This gradient was not seen in images of the other two bases. Moreover, the higher retained amount of FFA in the SC was confirmed by the MMP images and their fluorescence intensity.

In all tests, we used 80% DMSO as a positive control vehicle, and as DMSO was naturally toxic to the skin, we tried to find a base with positive effects on probe penetration and retention without any toxic effects.

Conclusion

Based on the findings of this study, TD Lipo showed a significant increase in the amount of FFA penetration and retention in the SC compared to other bases. These results were confirmed by MMP images, which showed that FFA had a lower fluorescence pattern in TD lipo, followed by a gradient of FFA concentration through the skin. However, a thorough comparison of the effects of these three commercially available transdermal bases on skin absorption and retention of formulated medicines must be done in the near future.

Recommendations

Use probes with different physiochemical characteristics. Examine the effect of pH and skin temperature on the amount of penetrated and retained probe.

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