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Betamethasone Dipropionate Loaded in Nanoliposomes vs Conventional Betamethasone Dipropionate: Comparative Study of Permeability and Penetrability *in Vitro* and *ex Vivo*

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Abstract

A betamethasone dipropionate (BD) liposomal cream was developed to treat rheumatological, inflammatory, allergic diseases and psoriasis. BD is a corticosteroid, anti-inflammatory, and immunosuppressant. However, adverse effects are associated with prolonged topical use. For this reason, liposomes were loaded with BD because they offer excellent biocompatibility, bio adhesiveness, and penetrability that improve the effects caused by the conventional drug. Liposomal dispersions were prepared by emulsification using phospholipid 90 (lipid) and Tween 80 (surfactant). The particle size, polydispersity index (PDI), and zeta potential were measured using a particle analyzer. The betamethasone (BM) percentage of encapsulated active (EA) ingredient was also determined through High Performance Liquid Chromatography (HPLC). The Franz cell and tape stripping characterized these *in vitro* and *ex vivo*. Then the final formulation reached a particle size of 70.80 ± 3.31 nm, a PDI of 0.242 ± 0.038, a zeta potential of −11.68 ± 0.77 mv and encapsulate active of 83.1% ± 2.4, complying with the parameters of a nanotechnological formulation. *In vitro* and *ex vivo* studies confirmed significantly efficacy of the cream over the commercial product, through the greater penetration into the pig ear skin, resulting in an improved drug. Finally, the liposomal cream demonstrated significant potential for enhanced percutaneous absorption, attributed to its nanometric size. This innovative nanotechnology approach aims to reduce the frequency of topical applications, thereby minimizing the side effects associated with psoriasis treatment.

Keywords

Nanotechnology, Betamethasone Dipropionate, Psoriasis, Liposomes, Drug Carrier Systems

1. Introduction

The skin helps the body defend itself against a vast majority of external aggressions and is one of the most important body's lines of defense. However, the strong barrier capacity of the skin is also a significant obstacle to the effectiveness of topical medications [1]. One of the skin diseases with the highest recurrence currently is Psoriasis (SP), a chronic inflammatory skin disorder mediated by the immune system, where the scalp is considered one of the most affected sites of the body during the disease [2]. This disease is rapidly becoming a serious public health problem around the world. The latest data presents a discouraging panorama of approximately 125 million affected people. Other data showed that the prevalence of this disease in the adult population varies from 0.9% in the USA to 8.5% in Norway. The 2016 reports from the World Health Organization (WHO) showed that in 1984, psoriasis affecting the population in China was 0.17%, and it increased to 0.59% after 25 years. Likewise, the situation in Europe, Spain, was 1.43% in 1984 and 2.31% after 15 years. National Health and Nutrition Survey reports also show an increase in disease prevalence from 1.62% to 3.10% between 2004 and 2010. It estimated that psoriasis treatment cost was about 11.25 million dollars in 2008. The annual expenses of American patients facing the psoriasis problem represent an average of 2528 US dollars [3]. In the same line, the situation in Latin America for 2016 was approximate: in Argentina 307,000, Mexico 202,000, Brazil 680,000, Colombia 60,000, Venezuela 49,000 and Chile 35,000 cases [4].

This disease presents a high recurrence and produces many adverse effects, such as hepatotoxicity, gastrointestinal inflammatory reactions, neutropenia, respiratory tract infection, nasopharyngitis, dry skin, fever, dyslipidemia, elevation of transaminases, hemoglobin reduction, thrombocytopenia, gastrointestinal perforation, and nausea [5].

Likewise, betamethasone dipropionate (BD), a salt derived from betamethasone (BM), is one of the most used corticosteroids in treating SP. Since BD has been marked as anti-inflammatory, immunosuppressive, and antiproliferative about epidermal cell turnover, specific alterations such as skin atrophy, stretch marks, rosacea, purpura, delayed wound healing, hypertrichosis, and altered pigmentation are among the adverse side effects associated with long-term BD (2). This corticosteroid is generally used as an anti-inflammatory, antiallergy, antiendotoxin, and immunosuppressive agent. According to the biopharmaceutical classification system (BCS), it is a type of BCS II drug that makes it almost insoluble in water. Therefore, clinical pharmacological forms of BM typically include betamethasone acetate, propionate, valerate, phosphate, and sodium phosphate [6].

Conventional therapy options, such as creams, ointments, and lotions, show limited accessibility to the deeper layers of the skin. A topical drug delivery system is widely used in various skin diseases. It is essential for efficient treatment due to the advantages of by passing the gastrointestinal tract, avoiding irritation of the gastric mucosa, the hepatic first pass effect, reaching directly to the injury, and then reducing unnecessary adverse reactions [1]. Therefore, to overcome all these deficiencies, new technologies based on nanotechnology have made it possible to develop lipid-coated drug delivery systems that increase flow through the skin and, thus, drug retention. Among the most widely used nanodrug delivery systems include lipid-based nanoparticles (NPs), *i.e.*, liposomes, ethosomes, nanoemulsions, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), lipid nanocapsules or nanocarriers based in polymers such as (polymeric NPs, polymeric micelles, polymer-drug conjugates) and peptide-drug conjugates (PDC) [7]. Therefore, it is necessary to develop new drugs with new technologies where the adverse effects produced by the active ingredients mentioned above can be improved. Therefore, tests must be developed with *in vitro* diffusion models, an important tool to evaluate the penetration capacity of active ingredients in various formulations. For transdermal administration, a reliable evaluation of the skin penetration-enhancing properties, the mechanism of action of the carrier systems and an estimation of bioavailability are essential [8].

The data mentioned above make this disease meaningful for the search for these innovative treatments. For this reason, in this study, a liposomal cream based on BM has been developed and compared in terms of diffusion and penetrability concerning a cream with a conventional formulation. Some techniques were developed, like Franz cells and tape stripping as *in-vitro* and *ex-vivo* diffusion models, respectively, to demonstrate the liposomal formulations advantages compared to the conventional drug, representing a possible novel therapy based on nanotechnology for psoriasis treatment.

2. Methods

In this study, we compare a novel topical formulation of betamethasone dipropionate (BD) in liposomes with a conventional formulation. A liposomal preparation with BD was made, and then characterized in a physical-chemical manner. Subsequently, the Franz Cell tests were performed to determine the permeability. Finally, the tape-stripping test was developed to obtain the penetrability of the new formulation, as reported by Sanabria *et al.* (2019) [9], with brief modifications.

2.1. Preparation of Liposomes

The liposomes were prepared using a mixture of surfactants, lipids and the active ingredient, in an aqueous solution of Glucose 0.5%. The aqueous and oil phases were combined with the active ingredient by mechanical agitation;

subsequently, both phases were combined by high-speed homogenization at a temperature of 80˚C, and finally, ultrasonicate, which allowed the spontaneous formation of lipid vesicles upon contact with the aqueous solution. This innovative process led to the active ingredient being trapped in the lipid bilayer, producing the liposomes, as reported by Sanabria *et al.* (2019) [9] with our brief modifications.

2.2. Determination of Particle Size, Polydispersity Index (Pdl), Zeta Potential, and pH of the Liposomal Dispersion

These physicochemical parameters were determined using dynamic light scattering at 25˚C on the zetasizer nano model ZS equipment from Malvern Instrument (USA). The autocorrelation function was analyzed using Nano-Zetasizer 7.11 software. It determines the particle size by averaging the rate of fluctuations in the intensity of laser light scattered by the particles as they diffuse into a fluid. This equipment operates at a wavelength of 632 nm and a maximum power of 10 mW [9]. The equipment is able to estimate the electrostatic interaction between the particles, thus indicating the stability of the dispersion [9]. This equipment uses the Laser Doppler Electrophoresis (LDE) technique that assumes the frequency variation of any type of wave emitted or received by a particle in motion. The sample used to determine particle size and polydispersity index was placed in a zeta cell, and potential measurements were made. It determines the particle size by averaging the rate of fluctuations in the intensity of laser light scattered by the particles as they diffuse into a fluid. The measurements were made as described by Sanabria *et al.* (2019) with brief modifications [9]. The dilution factor consisted of aliquots of 10 µL of the suspension in 990 µL of the dispersing phase (solution of glucose at 0.5%) for particle size, polydispersity index, and zeta potential). The measuring containers were plastic (particle size) and glass (zeta potential) cuvettes. A dip electrode was used to determine the zeta potential.

In addition, the physicochemical characterization of the liposomal cream was evaluated through the color, odor, appearance, PH value, and density. First, the pH value was determined at 25˚C using a pH meter brand GEHAKA, where the electrode was calibrated with standard buffer solutions of pH 4.0 and pH 7.0. The electrode was placed in the dispersion until stabilized. Then, the viscosity was determined using a Brookfield viscometer equipped with a suitable needle. Finally, the average of all determinations was made. Finally, all measurements were performing in triplicate, due to statistical reasons.

2.3. Determination of the Percentage of Encapsulated BM

The determination of the percentage of encapsulated active was developed through the molecular exclusion chromatography (CEM) method. The liposome sample was passed through a previously hydrated Sephadex G-25 column (pore diameter 50 - 150 µm) from the SIGMA brand. Subsequently, the sample was injected using perchloric acid + ammonium molybdate solution + Ascorbic Acid as the mobile

phase. Then, the elution were made using methanol to continue with the determination of the concentration by a high performance liquid chromatography (HPLC) technique. Then, 16 mg of the precipitate and the supernatant were weighed and the relevant measurements were made in triplicate, through HPLC equipment, model, XTERRA RP18 3.9 × 150 mm C18 column (10 × 300 mm), acetonitrile: water: methanol in a 58:32:10 proportion (mobile phase), 240 nm (detector wavelength), 1 mL/min (flow rate), 20 µL (injection volume) at 30˚C. Finally, the encapsulation percentage was expressed as a ratio between the area of the sample and the standard, with respect to the declared percentage of active of both the precipitate and the supernatant. In other words, the detection was at λ_{max} 254 nm. The formula used to calculate the percentage of encapsulated active (pellet) was the following:

$$\%\text{Encapsulated drug} = \frac{\text{total amount of ACV} - \text{Amount of unencapsulated ACV}}{\left(\text{total amount of ACV}\right)} \times 100$$

here the total amount of BD corresponds to the volume initially added in the nanodispersion formula (included in the pellet and supernatant) and the amount of non-encapsulated BD corresponds to the free drug found in the supernatant after centrifugation.

2.4. *In Vitro* BD Permeability Study—(Franz Cells Method)

For this study, diffusion assays were developed using the Franz cell method (Franz cells diffusion, model 58-001-430, Hanson Research, US) as described by Sanabria *et al.* (2019) [9], with modifications. A sample of 0.2 g of each of the 0.05% BD cream formulations (conventional and liposomal) was taken and then placed in the donor compartment of the Franz diffusion cell in triplicate. The dispersing medium consisted of phosphate buffer saline/methanol in a 70:30 proportion, respectively, and pH 5.8. A permeation profile was carried out at times 0, 1/2, 2, 4, 6, 22, 24, and 26 hours at 37˚C with a stirring speed of 1200 rpm. HPLC obtained the measurements in triplicate to make a statistical analysis. The permeation profiles were plotted as the diffusion rate of BD vs. time (Q, µg/cm^2) and as the percentage of BD released (%) vs. time. These data allowed us to find the maximum flow (J, µg/cm^2 h) permeated from the slope of the linear portion. The permeability coefficient (*Kp*) of the drug through the membrane was calculated using the relationship derived from Fick's first law of diffusion [9].

2.5. *Ex Vivo* BD Penetration, Study (Tape Stripping Method)

The tape removal technique is commonly used as a minimally invasive method to test the penetration of topically applied formulations through the stratum corneum, whereby layers of the stratum corneum are removed with an adhesive tape, and the residing layers of skin are examined. On the adhesive tape, this makes possible the quantification of APIs within the skin, which is crucial for topical and transdermal administration of drugs [10]. This study was developed using pig ear skin samples,

as it is a substitute for *in vivo* skin penetration studies, as it mimics human percutaneous penetration. In addition, pig ear skin shows similarities in morphology and penetration capacity and corresponds to human skin, making it the appropriate model for *ex vivo* skin penetration studies [8]. The penetration of the active ingredient into the different layers of the stratum corneum (SC) of the skin and the viable epidermis (VE) was then evaluated, as described by Sanabria *et al.* (2019) (9) with brief modifications, using Saarbrücken support. In this study, 1gr of BD cream (0.05%) was weighed, placed on the skin disc, and incubated at 37˚C for 2 hours. This assay was performed for triplicate. Subsequently, 20 viable epidermis (VE) tapes were collected and stored in 4 mL of 0.01 N NaOH solution for 24 hours at 25˚C. Then, HPLC analysis was developed under the conditions of section 2.4. Finally, the penetrability test of ACV in the stratum corneum was represented in a histogram by the relationship of the mass of ACV (μg) as a function of each layer (ribbon) and the EV. Data were statistically analyzed as mean ± standard deviation (SD) and by the student's t-test.

3. Results

The liposomal sample was white, unctuous, fast-absorbing cream. Presented a pH of 7.53 ± 0.02, within the physiological pH range, and a density of 1.02 g/mL ± 0.01. Using the zeta-sizer allowed the measurement of particle size, polydispersity index, and zeta potential, being these values: 70.80 nm ± 3.31, 0.24 ± 0.04, and -11.68 mV ± 0.77, respectively. After, with the help of the molecular exclusion separation method, the encapsulation percentage of BD in the emulsion was also determined, resulting in a high value of 83.1% ± 2.4 of encapsulated active, ensuring that a large part of the active ingredient was encapsulated within the vesicles formed. The following Table 1 summarizes the physicochemical characterization of the product.

Table 1. Physicochemical and organoleptic properties of liposomal test formulation.

Particle Size (nm)	70.80 ± 3.31
Polydispersity Index	0.24 ± 0.04
Zeta Potential (mV)	−11.68 ± 0.77
Density (g/mL)	1.02 ± 0.01
pH Value	7.53 ± 0.02
Encapsulated Drug (%)	83.1 ± 2.4

3.1. *In Vitro* Test (Franz Cell Diffusion) FCD

Comparing the release profiles of the two formulations tested, the test liposomal formulation obtained a higher diffusion rate: 3.46 μ/cm^2 ± 0.34 vs. the reference formulation, which had a 1.78 μ/cm^2 ± 0.30 (Figure 1). A higher percentage of the active ingredient was released in the liposome-based test formulation, achieving 6.09% vs. the reference formulation, which had 2.86% of permeation (Figure 2).

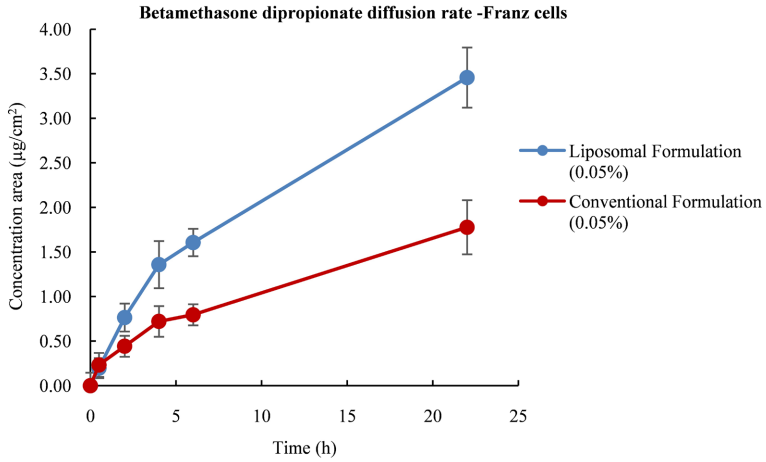

Figure 1. The diffusion rate of betamethasone dipropionate as a function of the time for the conventional formulation without liposomes versus the liposomal test formulation.

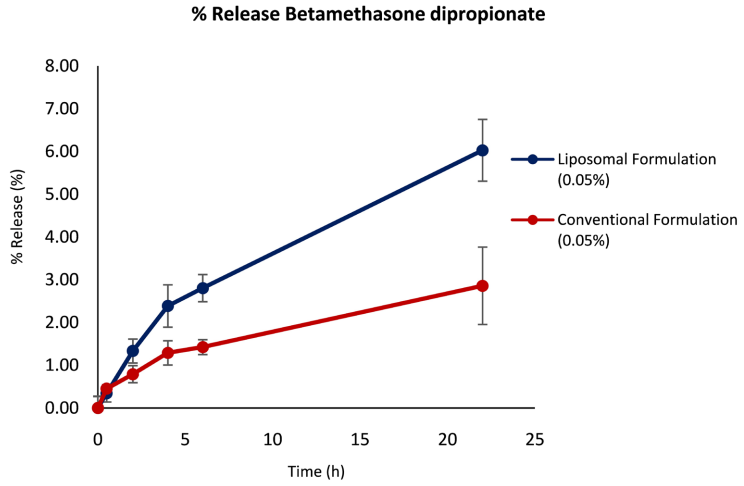

Figure 2. Percentage released of betamethasone dipropionate as a function of time for conventional formulation without liposomes versus test formulation with liposomes.

3.2. *Ex Vivo* Test (Tape Stripping)

Both formulations are present on the adhesive tapes tested, resulting in the liposome test formulation being the most frequently found in the SC and the VE. Then BD values were calculated for each adhesive tape; it was found that the average concentration of the liposomal formulation was higher than the conventional formulation (23.97 µg vs. 14.34 µg respectively). This allows us to deduce the total diffusion amount with the liposomal cream was 4.6 µg in comparison with the conventional cream, which was 2.4 µg. Data was obtained by adding the diffusion detected in the 10 layers and the viable epidermis.

Comparing the penetration, the differences between both formulations of them are clearly visible. Liposome test formulation not only managed to deliver the active ingredient to each layer of the SC studied, but also, when comparing the

average in micrograms of BD present in the SC and VE, we can see that the amount of the drug was greater than the conventional formulation (Figure 3). Data were expressed as mean ± standard deviation (SD). A statistical analysis was applied by the student's unpaired t-test. The value of $p < 0.05$ was considered a statistically significant difference.

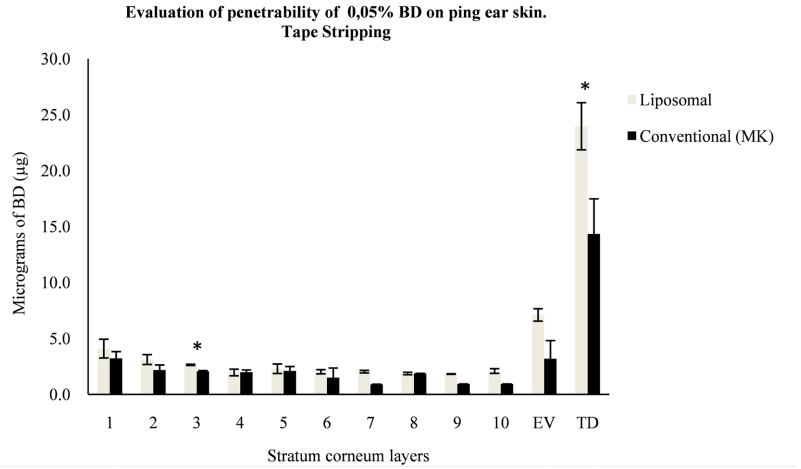

Figure 3. Comparison of the average micrograms of betamethasone dipropionate, present in the viable epidermis, between the conventional (MK 0.05% cream) vs. 0.05% liposome test formulations (mean ± SD; n = 2; *p > 0.05 significant).

4. Comparative Statistic of the *Ex Vivo* Test of Tape Stripping

Results were analyzed using the following data: in both drugs, conventional and liposomal formulation, comparisons were made based on the micrograms of BD present in the SC and the VE at the end of the experiment time. For them, the average or microgram average for each product was estimated as the median value, the standard deviation, the maximum and minimum microgram values of the active ingredient in each product, and the mass range for each one (Table 2).

Table 2. Comparative statistical analysis between Conventional product (BD MK 0.05% cream) and test (BD liposome 0.05% cream).

Variable	Conventional BD (MK) 0.05%	Liposomal 0.05%
Mean (µg of BD)	14.34	23.97
Median	2.1	1.9
Estándar deviation	2.11	3.14
Maximum value	7.9	4.34
Minimum value	1.8	1.8
Range (µg of BD)	6.1	2.54

Note. Statistical Analysis of the samples compared in the *ex vivo* assay based on the micrograms present in the SC and VE for the Conventional product (BD MK 0.05% cream) and test (BD liposome 0.05% cream).

It can also be said that because they are samples from different pharmaceutical formulations that are not related to each other, and second, these variables are quantitative, independent, and descriptive, it was necessary to perform independent means comparison tests. The physicochemical parameters of the 0.05% liposomal formulation confer a considerable advantage over the conventional formulation.

In both products tested (test liposome vs. conventional), comparisons were made based on the micrograms of BD present in the SC and the VE. In addition, the mass range for each of the formulations were estimated. The following table summarizes the values for each product.

Finally, to analyse a tape stripping assay was applied by the student's unpaired t-test. The value of $p < 0.05$ was considered a statistically significant difference.

5. Discussion

Reliable skin penetration data of active ingredients are indispensable in the pharmaceutical industry, particularly for drug delivery systems. This data directly impacts the active ingredients bioavailability, defined as the amount of molecules that reach systemic circulation [7].

This is why the success of topical drug delivery should depend on the ability to overcome biological barriers effectively. The skin is probably the most studied of such barriers. The skin's primary function is to limit the exchange of substances between the body and the environment. The skin barrier makes drug penetration a primary challenge in ensuring the efficacy of topical drug delivery in the skin [11]. The general principles governing drug penetration in the skin have been established. The stratum corneum is the rate-limiting barrier preventing dermal drug penetration. In other words, the rate at which a drug diffuses across the stratum corneum determines its overall dermal penetration and permeation rate [11].

In this context, dermal structure and organization are significant barriers to successful transdermal medication administration. The epidermis' multilayered structure and tiny pore size are a physical barrier to medication entry. Furthermore, the skin's highly lipophilic surface layer inhibits polar and charged molecules from entering, whereas the hydrophilic inner layer hinders the transfer of hydrophobic chemicals [12].

Due to this, lipid based nanoparticulate drug delivery systems are receiving growing attention. These systems, such as nanoemulsions, liposomes, niosomes, ethosomes, virosomes, ufasomes, and vesosomes, have the advantages of biocompatibility, well tolerability, reduced toxicity and increased bioavailability of poorly water-soluble drugs [13].

Therefore, the need to alter drugs and other ingredients on a nanometer scale through nanotechnological approaches has emerged, significantly enhancing the therapeutic outcomes of these materials and reducing the associated side effects. Surface area, site targeting, solubility enhancement, control delivery, and sustained release are a few properties that distinguish nanomedicines from conventional

counterparts [14].

This change in the active ingredient about the nanometric scale allowed the particle size, zeta potential, and EE of formulation three to be investigated. The results showed that BD particle size and zeta potential loaded in liposomes were 70, 80 nm, and −11.68 mv, respectively. Smaller particles (less than 200 nm) generally display more skin retention [15]. Zeta potential is a valuable sign in controlling the stability of BD, and an unstable system is usually accompanied by a decrease in the value of zeta potential, accelerating the approach of particles and formation of particle aggregation [16]-[19].

In this research, both *in vitro* and *ex vivo* methods were developed for detecting corticosteroid BD in the skin, comparing two formulations, a conventional cream and a novel liposomal, as we have already mentioned.

The industry standard method for evaluating these properties typically involves *in vitro* assays with porcine or cadaveric skin along with a Franz diffusion cell (Franz cell) as a surrogate for efficacy in living patients [20] [21]. As mentioned earlier, the Franz cell consists of a lower receiving chamber containing a receiving buffer with the solubility of the compound under study and an upper donor chamber where a test formulation can be applied to a skin sample mounted between the two chambers [21] [22].

In the same way, Pulsoni *et al.* (2022) mentioned that the FDC system could be either a static or a flow-through setup. The receptor compartment is filled with a physiological buffer solution, where the compound is released after penetrating through the skin surrogate. Onto this surrogate, a finite (≤10 μL/cm^2) or infinite (≥10 μL/cm^2) formulation dose can be topically applied into the donor compartment and allows the evaluation of penetration kinetics over time patients [7].

Through the chamber, a conventional active ingredient and another with nanometric characteristics were passed, which could cross the synthetic membrane through a passive diffusion process from the donor compartment to the recipient compartment that contained the PBS buffer pH 5.8:methanol (70:30). Subsequently, when evaluating the curves of the release profiles obtained (**Figure 3**), it was found possible to observe that BD managed to diffuse faster in time 22 hours of as a function of the gradient, concerning the conventional formulation, observing essential differences between the test formulation and the reference. In our study, with the liposomal formulation, 6.09% diffusion was achieved in comparison with 2.86% with the reference formulation, similar to other researchers [2] [13] [23].

We can observe how the inclusion of BD into liposomes notably increases its permeation levels compared to the commercial formulation. It makes evident that the cumulative amount of drug that penetrated through the membrane after 22 h was greater in the liposomal formula than in the conventional formula [23] [24]. This permeation of the active principle concerning the area increases in the designed nanostructured preparation; it was necessary to determine if the percentage of active ingredient released corresponded to this observed phenomenon and

to give even more strength to the previous results [24].

This evidence shows that the liposomal active ingredient presents greater permeation, passing more from the donor compartment to the recipient, achieving superior behavior compared to the reference formulation due to nano-liposomal characteristics.

Trying to explain why liposomes increase the permeation of BD through the synthetic membrane into diffusion cells, we found that liposomes have emerged as the pioneering nanocarriers for drug delivery across various administration routes, including skin penetration [25]. Liposomes are versatile colloidal particles comprising different phospholipids, cholesterol, and an aqueous medium. Commonly the most used natural phospholipids (PLs) are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidyl inositol (PI), and phosphatidyl glycerol (PG), are preferred for their cost effectiveness and favorable toxicological profiles. In addition, they exhibit several advantageous characteristics, including biocompatibility, biodegradability, nonimmunogenicity, and recognition as generally recognized as safe (GRAS) components by the FDA. These attributes make phospholipid-based vesicles, *i.e.*, liposomes, highly suitable as drug nanocarriers [25]. Liposomes can encapsulate hydrophilic drugs within their aqueous compartments and incorporate lipophilic drugs within their lipid bi-layers. Additionally, liposomes can enhance the solubility of poorly water-soluble drugs, which is important since most drugs used for the treatment of acne are not hydrophilic, as well as to improve the chemical stability of drugs, particularly photostability [25].

Similar results were obtained by Yu Y-Q *et al.* (2024), where they showed that increasing the percent of the lipid in the formulation has increased the capability of drug penetration to/through the skin by aiding the penetration of the drug through the stratum corneum. This suggested that nanocarriers containing 30% more lipids could be used for transdermal absorption of the drug, while formulations containing only 10% the lipids could be recommended for localizing higher amounts of betamethasone dipropionate in the skin layers as compared to traditional cream [14].

Another innovative topical ointment containing betamethasone dipropionate loaded nanostructured lipid carrier for the treatment (BDNLC) of atopic dermatitis was developed. Researchers found desirable drug retention in the skin tissue of living mice and no skin irritation in rabbits. In conclusion, this study will facilitate the development of BDNLC, which may improve skin retention, reduce the adverse effects induced by systemic absorption, and reduce skin irritation [26].

Therefore liposomes have found significant applications in dermal and transdermal drug delivery, including their targeted delivery to skin appendages. In the dermal drug delivery context, liposomes enhance drug penetration into the skin, ensuring localized therapeutic levels while minimizing percutaneous absorption. Liposomes contribute to improved therapeutic efficacy by reducing side effects and serve as a local depot for the sustained release of dermally active components.

The inclusion of the betamethasone into the lipid bilayer versus the aqueous compartment through, for example, the use of cyclodextrin complexes seems to be the best choice as it gives rise to higher entrapment efficiencies [25].

It was also reported that SCL (stratum corneum lipid) liposomes had a higher skin deposition than o/w emulsion and hydro-alcoholic drug solutions [26]. The other advantage of liposomes as topical drug delivery systems is their potential follicular targeting [27]. Liposomes can also provide transdermal drug delivery, and recent reports show that deformable vesicles (called transfersomes) are a better choice than conventional liposomes for transdermal delivery purposes [28]. In addition, the addition of surfactants to liposome composition results in more flexible and elastic lipid bilayers, which can, in turn, significantly increase drug permeation through the epidermis and transdermal drug delivery [28].

In this context, skin is a strong barrier toward many drugs administered via the topical route. The stratum corneum is the strongest barrier in the skin and is responsible for limiting drug penetration through skin layers. In addition to stratum corneum, drugs must overcome other cellular and molecular barriers such as antimicrobial barrier, Langerhans cells in the epidermis layer, macrophages in the dermis layer, dendritic cells, and enzymatic systems. Drug molecules fate during skin penetration across the stratum corneum [29]. For this reason, in recent years, nanoparticles have been highly considered as a permeation enhancing strategy to overcome the barrier characteristics of the different layers of the skin. On the other hand, targeting the different skin organelles, including the pilosebaceous gland, hair follicle, and dermis layer, for the better management of different local diseases of the skin layers more considered during the last decades [29].

Also, is important to mention, that to obtain reliable dermal permeability data, several parameters have to be considered for the test design system, which are influenced by the solubility of the compounds: the sink condition, the incubation time, the incubation temperature, the mixing, the hydration of the membrane, the amount of dose. All of them are critical parameters and play and important role in methods to evaluate of skin penetration [10].

In this research, we also developed an *ex vivo* tape stripping, this It is defined as the process in which successive layers of the stratum corneum are removed by traction on the surface of the skin with an adhesive substance [30] [31]. In studies of this nature, pig ear skin represents an appropriate model *ex vivo* to predict the behavior of a topical administration system, because it has histological and structural similarities with human skin. In animal models it is estimated that pig ear skin is the one with permeability characteristics more similar to human skin [32] [33]. besides being considered as a study model for topical absorption (Epicutaneous or transdermal) of drugs for Neonates [34].

The present assay, explored the diffusion of BD liposomal formulation compared to the reference formulation. We observed that, throughout all the 20 evaluated layers according with Sanabria *et al.* (2019) [9] whit brief modifications, significant quantities of both formulations are found only in the first 10 layers,

these quantities were negligible from layer 11 onwards, which is why they were not graphed. However, a greater presence of active ingredient is seen in VE layer evaluated, but in a higher content of the liposomal formulation. The total BD values achieved through the SC layers and the skin by the liposomal formulation was statistically higher than that of the conventional one ($p > 0.05$).

Therefore, can be inferred, this nano-constructed formula managed to provide a greater amount of drug in the remaining skin, also in VE compared to the conventional formulation. Different research groups obtained similar results when they use BM liposomal preparations [2] [13] [24].

A possible explanation for this phenomenon is that the staggered corneocyte arrangement in a lipid continuum is suggested to confer a highly twisted lipoidal diffusion pathway making it 1000 times less permeable to water than other biomembranes. The transport role of this twisting pathway is further elucidated by visualization studies including localization of different permeants in the intercellular channels [14] for this, the design of superloaded formulation like liposomal systems, is the more innovative approach to overcome the skin barrier. These approaches have modestly improvement on the penetration across the membrane but are unlikely to transform an impermeable drug entity into an ideal transdermal candidate [17] [31].

Other important aspect, in the case of topically applied liposomal formulations, is the use of *ex vivo* models employing viable human skin is being developed to fill the gap between in vivo results obtained by animal models and possible behavior after administration in man [35]. Despite the system's higher complexity, defined as isolated perfused human skin flap (IPHSF), the last decade witnessed an increase in its use, also for testing lipid based nanosystems [36]. The knowledge on the fate of the drug carried by nanocarrier in these models can help the design of the clinical trials mitigating the risk of their failure in terms of either efficacy or safety [37].

For these reasons, the best penetration will depend of the physicochemical parameters of the active ingredient (data present in **Table 1**), where the drug presented a particle size less than 100 nm, low polydispersity index, which indicates; a homogeneous particles size distribution and positive zeta potential, conferring stability to the liposomal preparation. It favors the penetration compared to the large molecules of the conventional drug. From this evidence, we can infer that modifying the drugs at the level of their structural lipids enhances the permeation of the active ingredient, facilitating the penetration.

6. Conclusion

This work evidenced the potential of liposomes as carriers for topical delivery of betamethasone. The use of liposomes may represent a promising approach in psoriasis treatment. In this sense, based on the previous discussion, it can be concluded that the test formulation (BD liposomal 0.05% cream) is superior to the conventional formulation (MK® 0.05% cream), in terms of diffusion and penetration at

the level of the stratum corneum of the skin. The technique mainly used was based on hot homogenization followed by sonication; also, a factor that had an important role in the determination of physicochemical properties was the choice of the surfactant tween 80, phospholipon as lipid phase that allowed the improvement of the liposomal dispersion. Betamethasone liposomes acted as a highly efficient drug carrier and as a system that facilitated the delivery and permeability of the active ingredient through synthetic and natural membranes. Furthermore, the nanodelivery system used was capable of transporting the betamethasone drug through the skin, reaching deeper layers, maintaining its size and charge, and optimizing its release at the target site of the skin where psoriasis manifests. The BM liposomes prepared by nanotechnology in Laboratories Leti provide the bases for large-scale production in the future and indicate the direction of research and development of new BM dosage forms.

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Ethical Approvals

This study does not involve experiments on human or animal subjects.

Conflicts of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

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