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# Liposomes Carrying Diclofenac Diethylammonium: A Penetrability and Permeation Study

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## Abstract

This study investigated whether liposomes could enhance the permeation and penetration of diclofenac diethylammonium. For this, a 1.16% diclofenac diethylammonium liposome gel formulation was developed (Grupo Leti, S.A.V.). *In vitro* and *ex vivo* tests were conducted to analyze the diffusion and penetration profiles of the formulation. The profiles obtained were compared with a commercially available product, DiAnalper gel (Pharmetique Labs). The *in vitro* test was assessed in a Franz diffusion cell system using a dialysis membrane. The cumulative amount of drug permeated after 24 h demonstrated a significantly ($p < 0.05$) enhanced diffusion for the liposomal gel formulation compared to the commercial product. Specifically, the liposomal gel exhibited values of 710.56 ± 12.23 μg/cm$^2$, whereas the commercial formulation yielded values of 371.00 ± 3.54 μg/cm$^2$. These findings were further supported by consistent results in the percentage of drug release, flux, and permeability coefficient, all indicating a notable improvement in diffusion associated with the liposomal gel formulation. The tape stripping assay performed on pig ear skin demonstrates a statistically significant difference ($p < 0.05$) between the penetration transport of the diclofenac from liposome gel formulation (1413.95 ± 250.51 μg) and the conventional product (202.36 ± 18.07 μg) the liposomal formulation was able to cross de stratum corneum and deliver a high amount of drug to the skin. These findings demonstrated that incorporating diclofenac into a liposomal system significantly improved the drug delivery, which could confer an advantage for clinical uses.

## Keywords

Liposomes, Diclofenac Diethylammonium, Transdermal Drug Delivery, Skin Permeation, Skin Penetration

## 1. Introduction

Transdermal administration provides unique benefits as compared to other routes for drug delivery. It allows the drug to be transported directly through the different layers of the skin until reaches the bloodstream, reducing systemic exposure and the risk of side effects associated with oral administration [1]. During the development of a transdermal formulation, the main challenge is the low permeability of the stratum corneum, the outer layer of the skin, which limits the range of active ingredients that can be used. Different strategies have been employed to improve drug penetration, including the use of encapsulation systems such as liposomes. Liposomes are spherical vesicles with an aqueous core surrounded by one or more lipid bilayers. Due to their structure, liposomes can transport pharmacologically active hydrophilic or hydrophobic products inside the aqueous core or between the lipids. The interaction of liposomes with the skin and subsequent transdermal diffusion depends on several physicochemical factors of the nanocarrier, such as vesicle flexibility and charge, as well as the structural conditions within the skin and the environment [1] [2]. Liposomes serve as penetration enhancers by diffusing into the stratum corneum leading to the disruption of bilayer fluidity. This process loosens the lipid arrangement within the stratum corneum, thereby compromising the barrier function of these layers and facilitating drug delivery [1].

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used as the first-line therapy for managing pain and inflammation due to their ability to inhibit the synthesis of prostaglandins, prostacyclins, and thromboxanes by suppressing the activity of cyclooxygenase (COX) [3] [4]. Within this family, diclofenac, a phenylacetic acid derivative, is present on the market in its acid or its ionic forms, such as sodium, potassium, or diethylammonium. Topical formulations containing sodium or diethylammonium salts at 1% - 3% concentrations commonly treat localized musculoskeletal conditions. The active ingredient penetrates the skin barrier, reaching the joints, muscles, and synovial fluid; it is preferentially distributed and persists in the inflamed tissues, where it exerts its local therapeutic activity [4]. Regarding the properties of diclofenac diethylammonium (DEA) and the ability of liposomes to improve drug penetration, in this study, we aimed to develop a cream formulation containing 1.16% diclofenac diethylammonium loaded in liposomes (DEA-L, Grupo Leti S.A.V) and compare its diffusion and penetration profiles with a reference product available on the market (DiAnalper gel, Pharmetique Labs). The study investigated whether liposomes could enhance the drug's penetration and availability by conducting i*n vitro* and *ex vivo* tests to compare the formulations.

## 2. Materials and Methods

### 2.1. Preparation of a 1.16% Diclofenac Diethylammonium Liposome

Liposomes were prepared by sonication using a mixture of soy lecithin, surfactants, co-solvents, and diclofenac diethylammonium in a glucose solution. The components were incorporated by mechanical agitation. After the dispersion process,

various excipients were incorporated to achieve the desired physicochemical characteristics of the galenic formulation.

## 2.2. Characterization of the Liposome Suspension

### 2.2.1. Particle Size, Polydispersity Index, and ζ-Potential

The hydrodynamic size and the polydispersity index (PDI) of the liposomes were determined by dynamic light scattering using a Zetasizer Nano ZS (Malvern, USA) with a red laser $\lambda$ = 633 nm, as described by [5] with minor modifications. The autocorrelation function was analyzed using Zetasizer 7.11 software. For the measures, polystyrene disposable cuvettes were used. The liposomal dispersion was diluted 1:100 in a 0.5 % glucose solution. The reported size and the PDI were based on the intensity results. The results represent the average of three sample measurements taken at 25˚C after 20 sec of equilibration time.

The ζ-potential was measured using the same instrument. The liposomal dilution (1:100 in a 0.5 % glucose solution) was measured in a universal dip cell using Smoluchowski's equation. The results represent the average of three sample measurements taken at 25˚C after 20 sec of equilibration time.

### 2.2.2. Determination of the Percentage of Encapsulated Active Ingredient

To determine the percentage of encapsulated active ingredient, the liposomal dispersion was centrifuged at 13,500 rpm for 20 minutes at a temperature of 4˚C using an SS-34 fixed-angle rotor. The obtained fractions underwent analysis via high-performance liquid chromatography (HPLC) equipment (Alliance, Model 746, Waters, US) according to the method previously recorded [5] with modifications. Briefly, the samples were prepared in MeOH/water (7:3) medium. An amount of 10 µl was injected in an XTerra RP-18 column using phosphate buffer pH 2.5/methanol (3:7, v/v) as a mobile phase. Elution was conducted at 25˚C, with a flow rate of 1 mL/min, 2000 psi, and monitored at 254 nm UV-detector.

Equation (1) was used to calculate the percentage of encapsulated active:

$$\%\mathrm{EE} = \frac{\left(\text{total amount of DEA} - \text{Amount of non encapsulated DEA}\right)}{\left(\text{total amount of drug}\right)} \times 100 \quad (1)$$

The total amount of DEA corresponds to what was initially added in the nanodispersion formulation (included in the pellet and supernatant), and the amount of non-encapsulated DEA corresponds to the free drug found in the supernatant after centrifugation.

## 2.3. *In Vitro* Permeation Using Franz Diffusion Cells

For this study, diffusion assays were conducted using vertical Franz cells (Hanson Research, Model 58-001-430), as described by [5], with brief modifications. DEA-L was compared to a conventional no-liposome diclofenac diethylammonium formulation (DiAnalper gel from Pharmetique Labs). The capacity of the receptor chamber was 7 ml, and the inner diameter was 15 mm. A SIGMA brand dialysis membrane of 12,400 Da porosity, previously activated by washing with aqueous

solutions of sodium sulfide, sulfuric acid, and water as described in the supplier's product information sheet [6], was fixed between the donor and the receptor chamber. The membrane area in the Franz cell was 1.77 $cm^2$. The receptor medium consisted of a mixture of saline phosphate buffer (pH: 7.4)/methanol (70:30) previously sonicated and degassed. Before starting the diffusion experiments, the system was equilibrated at a temperature of 37˚C, which was maintained throughout the process. The stirring speed inside the receiver cell was adjusted to 1200 rpm. Next, 0.2 g of each of the formulations under study was placed in the donor compartments of the cell, and samples were taken in triplicate at times 0, 0.5, 2, 4, 6, and 24 hours. The volume taken was immediately refilled with fresh solution. The samples were analyzed in triplicate by HPLC. The conditions used were similar to those described above, except for the volume injected, which was 50 µl. Permeation profiles were plotted as the cumulative release of the drug (Q, µg/$cm^2$) vs. time (h) and the percentage (%) release as a function of time. The maximum flux (J) was derived from the slope, and the unit is µg/$cm^2$/h. The permeability coefficient ($K_p$) across the membrane was calculated using a relation derived from Fick's first law of diffusion, which is expressed in Equation (2):

$$K_p = \frac{J}{C} \tag{2}$$

where $J$ is the flux, and $C$ is the drug concentration in the donor compartment.

### 2.4. *Ex Vivo* Penetration Test

The penetration of the active ingredient into the different layers of the stratum corneum was evaluated using tape-stripping tests performed on pig ear skin, which has been reported as an appropriate model for *ex vivo* studies due to its histological and structural similarity to human skin [7]. For this, the procedure described by [5] was followed, with brief modifications, for each formulation. Skin disc sizes were 1.5 cm, and each formulation, DEA-L, and DiAnalper gel was dosed with 0.5 g in duplicate. A total of 20 layers were removed using adhesive tape after the sample was incubated for 2 h at 37˚C. The tapes and the skin were collected in Falcon tubes to which 5 mL of methanol was added. The samples were analyzed by HPLC in triplicate using phosphate buffer pH 2.5/methanol (3:7, v/v) as a mobile phase delivered at 1 ml/min at 2000 psi. The amount of sample injected was 50 µl, and it was monitored at 254 nm UV-detector. The penetrability of DEA-L in the pig ear skin was plotted in a histogram using the ratio of DEA-L mass (µg) as a function of each layer (tape) and the skin.

### 2.5. Statistical Analysis

Data analysis was carried out using Microsoft Excel 2016. Results are expressed as mean ± standard deviation (SD). Statistically significant differences were determined using the student t-test. A probability of $p < 0.05$ was considered statistically significant.

## 3. Result and Discussion

The therapeutic efficacy of a topical drug depends on its ability to penetrate the skin and reach the target. Several scientific studies have documented an enhancement in the transdermal delivery of pharmaceuticals through the skin's outermost layer, the stratum corneum (SC), using carriers such as liposomes [1] [2]. The SC presents a bottleneck for drug permeability, thereby limiting the range of possible pharmaceuticals [1] [2]. Diclofenac diethylammonium is widely employed for its anti-inflammatory and analgesic properties. To achieve optimal effectiveness, this compound must penetrate the skin barrier at an appropriate concentration to exert its intended therapeutic effects [3] [4]. Given the inherent variability in transdermal absorption of diclofenac and the promising potential of liposomes in enhancing permeability, our current study seeks to formulate a cream containing 1.16% diclofenac diethylammonium encapsulated in liposomes. Subsequently, the release and permeation profiles of the developed formulation were compared with those of a conventional diclofenac diethylammonium product available on the market.

### 3.1. Characterization of Liposomes

The physicochemical properties of liposomes are determined by their composition and the preparation methodology. Key parameters such as size, PDI, $\zeta$-potential, and encapsulation efficiency have been carefully measured, and the findings are outlined in **Table 1**.

**Table 1.** Properties of the 1.6% diclofenac diethylammonium liposome dispersion.

| Particle Size (nm) | PDI | $\zeta$-potential (mV) | % EE ± SD (%) |
|---|---|---|---|
| 40.0 | 0.70 | −16.60 | 92.40 ± 1.40 |

The prepared liposomes reveal a mean size below 100 nm, consistent with that observed in previous studies [8]. It has been demonstrated that diclofenac, like other non-steroidal anti-inflammatory compounds, has surfactant properties due to its amphiphilic nature. As a result, it self-associates and binds to membranes, causing partial disruption and solubilization [8]-[10]. The encapsulation efficiency was found closed to 100%, indicating that a large part of the active principle is encapsulated within the liposomes. This result was similar to that obtained for elastic niosomes loading DEA [11], vesicles made of a mixture of phospholipids, surfactants, ethanol, and the active ingredient. The higher percentage of encapsulation in these elastic niosomes is related to the improved solubility of diclofenac diethylammonium by the ethanol that facilitates the entrapment of the drug within the vesicle [11] [12]. Similarly, in our development, a non-ionic surfactant and an alcoholic co-solvent were used.

### 3.2. *In Vitro* Permeation through a Membrane

The Franz cell model determines the diffusion of compounds through the skin or artificial membranes, providing an alternative approach to addressing safety and

effectiveness concerns in the development of dermatological products [13]. Skin permeation is majorly governed by Fick's Law, which states that the flux (*J*) or absorption rate of any substance across a barrier is related to its diffusion coefficient, which in turn is directly proportional to the concentration gradient. The diffusion coefficient is influenced by several factors, including the physicochemical properties of drugs, barriers, interactions between drugs and skin lipids, as well as the composition of semi-solid dosage forms [14].

The *in vitro* release profile of DEA-L and DiAnalper gel formulations showed a superior diffusion of liposomal diclofenac compared to the conventional product (**Figure 1** and **Figure 2**). The cumulative amount of liposomal and the conventional product permeated through the cellulose membrane progressively increased. For all points, the amount released per surface area of the liposomal diclofenac was approximately two to five times more than the conventional product (**Figure 1**). The statistical analysis demonstrated that the cumulative amount of the drug permeated from the liposomal formulation was significantly higher than that from the conventional product for each measurement ($p < 0.05$, **Figure 1**).

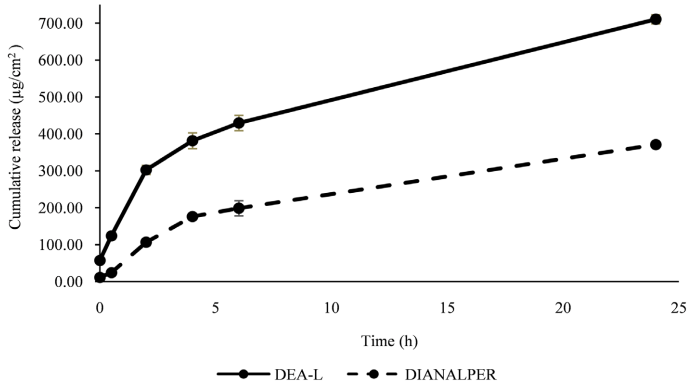


**Figure 1.** Diffusion rate of diclofenac diethylammonium as a function of time for the conventional formulation without liposomes (DiAnalper) versus test formulation whit liposomes (DEA-L). The measures were performed through a dialysis membrane in Franz diffusion cell (mean ± SD; n = 3; $p < 0.05$).

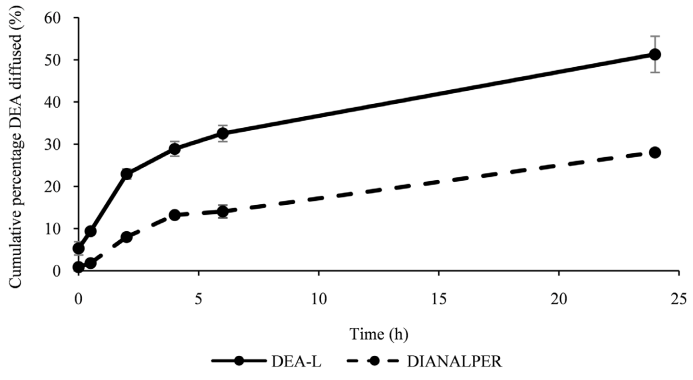


**Figure 2.** Percentage release of diclofenac diethylammonium as a function of time for conventional formulation without liposomes (DiAnalper) versus test formulation whit liposomes (DEA-L). The essay was conducted using a Franz diffusion cell with a dialysis membrane (mean ± SD; n = 3; $p < 0.05$).

The amount of drug permeated after 24 h for liposomal formulation exhibited values of 710.56 ± 12.23 μg/cm$^2$, whereas the commercial formulation had 371.00 ± 3.54 μg/cm$^2$. Similarly, we obtained significant differences by analyzing the percentage of the drug release from the liposomal and the conventional formulation ($p < 0.05$, Figure 2). These findings indicate that the liposomal system improves the penetration of diclofenac diethylammonium. Comparable conclusions could be obtained by analyzing the maximum flow rate and the permeability coefficient (Table 2). Thus, the liposomal formulation not only permeated faster but also around half of the active ingredient placed in the receptor compartment penetrated into the donor chamber, achieving superior performance concerning the reference formulation due to its nano-construction.

**Table 2.** Permeated amount of diclofenac diethylammonium at 24 h, flux, and permeability coefficient.

| Formulation | Permeated amount at 24 h ($\mu$g/cm$^2$) | $J$ ($\mu$g/cm$^2$h) | Permeability coefficient ($K_p$) × 10$^{-3}$ (cm/h) |
|---|---|---|---|
| DEA-L | 710.56 ± 12.23 | 23.46 | 2.02 |
| DiAnalper | 371.00 ± 3.54 | 13.71 | 1.18 |

The permeated amount is described as mean ± standard deviation (n = 2).

The kinetic release mechanism was assessed by analyzing the permeation data using the zero-order, Higuchi diffusion, and Korsmeyer-Peppas models. The coefficient of determination ($R^2$) was used as the basis for comparison. Both formulations showed a good fit with the Higuchi diffusion model ($R^2 > 0.9$). This result confirms that a diffusion process following Fick's law governs the release.

As previously mentioned, the diffusion is influenced by several factors. Essentially, a dialysis membrane can be considered a sieve, which separates the sample from the receptor medium based on the size of the molecules. Therefore, the ability of an active ingredient to penetrate may depend on its solubility, as well as the volume and size of the active substance [11] [12] [14] [15]. Consequently, for this system, it can be inferred that the smaller size of the liposomes and their affinity for the receptor medium results in a faster diffusion rate and higher drug diffused compared to the conventional product. Furthermore, it is important to emphasize the critical role that certain excipients play in the solubility of the drug formulated. It has been reported that including alcohol solvents and a surfactant probably enhanced the solubility and diffusion of DEA in an aqueous medium and imparted elasticity to liposomes [11] [12] [15]. For instance, the findings obtained by [12], who conducted an *in vitro* study to compare the drug release and diffusion of four different formulations of diclofenac: a) diclofenac aqueous gel containing micelles, b) diclofenac lotion, c) diclofenac lipogel, and d) the commercial emulgel Voltaren®. They found that the diclofenac lotion, formulated with soy lecithin, ethanol, and buffer that may contain ethosomes, exhibited the highest drug release and diffusion, and they suggest that the ethanol acts as a permeability enhancer. Reference, [16] compared *in vitro* diclofenac potassium release using a dialysis

bag from pure diclofenac, liposomes, and bilosomes, which are deformable vesicles, composed of phospholipid, surfactant, cholesterol, and bile salt. A significant high release of the drug was achieved from bilosomes and liposomes. The release from bilosomes was approximately three times higher than pure diclofenac due to an enhancement in drug solubility and the inclusion of bile salt. Hence, these studies enable us to suggest that the different features of the liposome cream developed (vehicle used, size of the construct, solubility enhancers, etc.) are the ones that generate a significant difference from the conventional diclofenac.

### 3.3. *Ex Vivo* Penetration Across Porcine Skin

Monitoring the delivery of a drug into the skin is a crucial step for developing a formulation. The process of approval of a drug formulation requires the demonstration of bioavailability and bioequivalence [7]. To address this, *ex vivo* or *in vivo* studies must be conducted. The tape-stripping technique is a widely accepted methodology for examining the localization and distribution of substances within the SC [7]. Porcine skin has been recognized as a suitable model tissue for predicting human skin permeability despite having a lower barrier function than human skin [17].

The tape-stripping study showed that both formulations are present in the different layers that constitute the SC and the skin (**Figure 3**). The total DEA amount estimated from the 20 tape strips was 248.42 ± 33.20 μg for DEA-L and 179.44 ± 15.82 μg for DiAnalper. In the skin, the amount of DEA was significantly higher ($p < 0.05$) when using the liposomal condition compared to the conventional product (1413.95 ± 250.51 μg and 202.36 ± 18.07 μg, respectively). These findings suggest that liposomal diclofenac may be more effective because more drugs have reached the skin. As a result, it may penetrate in larger quantities into the underlying tissues to exert its action at the site of pain and inflammation.

It is well known that the absorption and subsequent penetration of a drug into deeper tissues is a complex process limited by various factors, such as the drug's molecular size, lipophilicity, and protein/tissue binding capacities [18] [19]. Literature reported that liposome formulations enhance skin absorption by diffusing into the SC, disturbing the lipid structure, and modifying the structure of the intercellular lipid lamellae [15]. Several mechanisms have been described for the permeation of liposomes through the skin. Conventional liposomes, composed of natural or synthetic phospholipids and potentially incorporating additional lipids such as cholesterol, can engage in several interactions with the skin. These interactions include: a) adsorbed to the skin surface with a subsequent release of the drug to the skin, b) fused with the lipid matrix of the SC, increasing the diffusion of the active within the skin, and c) exchanging lipids between the liposomal membrane and the cell membrane, facilitating the diffusion of the drug across the membrane [20] [21]. Although conventional liposomes can interact with the skin and increase the permeation of the drug, they cannot cross the SC intact [20] [21]. In contrast to conventional liposomes, deformable liposomes—such as ethosomes,

transfersomes, niosomes, and bilosomes—are typically formulated with the addition of surfactants, alcohols, or compounds like bile salts. These liposomes serve as effective drug delivery vehicles, exhibiting high membrane flexibility that enables them SC while maintaining their structural integrity. This enhanced flexibility allows the liposomes to "squeeze" through skin pores or constrictions that are significantly smaller than their own size [20]-[22].

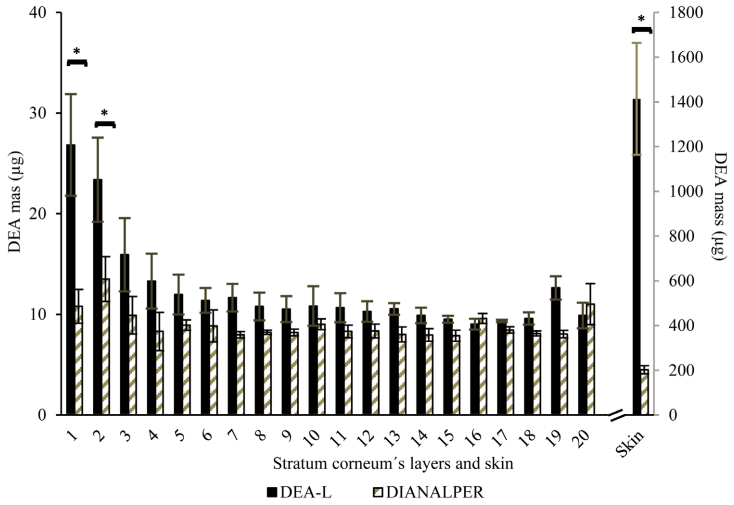


**Figure 3.** Comparison between the average amount penetrated of conventional formulation without liposomes (DiAnalper) and the average amount penetrated of the test formulation whit liposomes (DEA-L) in the layers of the stratum corneum and the skin (mean ± SD; n = 3; $^{*}p < 0.05$).

After analyzing the results of the *ex vivo* test shown in this paper (**Figure 3**), we infer that both permeation mechanisms may be taking place simultaneously, with the deformable liposomes mechanism possibly predominating over the conventional mechanism due to the physical-chemical properties of the liposomes. Consequently, it is plausible that the liposome formulation may accumulate within the initial 2 - 3 layers of the SC, where it undergoes a transformation. This transformation potentially facilitates its travel through the subsequent layers. As evidenced by the results from the Franz cells (**Figure 1**), this could lead to a more substantial and possibly more rapid migration of DEA-L. Conversely, the conventional formulation seems to permeate steadily through the various layers of the SC, consistently maintaining lower quantities than those observed with the liposomal formulation. This behavior may be attributed to its slower passage, likely due to greater restriction, resulting in limited penetration into the skin.

In this regard, the deformability of liposomes containing DEA might be attributed to their formulation with phospholipids, surfactants, and alcoholic solvents, which are also recognized as penetration enhancers [11] [12] [15] [20]. Including a surfactant in the liposomes facilitates their deformation without rupture, thereby rendering the phospholipid bilayer more fluid and flexible [20]. Furthermore, alcohol molecules promote the interdigitation of phospholipids, reducing

packing density and increasing malleability [20] [22]. Consequently, this combination of components leads to the formation of small vesicles with a high curvature radius that destabilizes the phospholipids. This induces a redistribution of amphiphilic molecules, ultimately leading to an increase in the flexibility of the lipid bilayer [20] [23]. This type of liposome generally demonstrates high encapsulation efficiencies [11] [20]-[22]. Therefore, these findings could contribute to explaining the results shown in this study.

Similar studies about the effect of liposome parameters and composition on transdermal transport of diclofenac have been published. Reference [15] compared the *ex vivo* transdermal transport of three topical formulations of diclofenac incorporated in a carbopol matrix: conventional liposomes, ethosomes, and transfersomes, through rat skin. They found that the flexible vesicles, transfersomes and ethosomes, provided a significantly higher cumulative permeation, flux, and permeability coefficient into the skin than the conventional liposomes, conventional gel or hydroethanolic solution. Reference [24] investigated transdermal transport of a 1% liposomal diclofenac sodium gel across human skin and comparing with two emulsion gel formulations from the market: Voltaren® Schmerzgel 1.16% and Voltaren® Schmerzgel forte 2.32% (equivalent to 1% and 2% diclofenac sodium, from Novartis Consumer Health GmbH, Germany). The liposomal diclofenac gel enhanced the *ex vivo* transport compared to their equivalent market product (Voltaren® 1.16%). More recently, the investigation of [25] showed phenomenological dynamics similar to our findings in the *in vitro* experiments when comparing two commercial diclofenac sodium formulations, namely Diclac® Lipogel (Sandoz Pharmaceuticals, Basel, Switzerland) that is a liposomal base formulation, and Primofenac® Emulsion gel (Streuli Pharma) across human skin. Furthermore, the tape-stripping studies showed that Diclac exceeds Primofenac in terms of promoting the delivery of the drug to the skin.

Since several investigations have shown concordance between *ex vivo* and *in vivo* assays [25]-[27], this study's results revealed the potential for clinical use of diclofenac-loaded liposomes with a size of less than 100 nm.

## 4. Conclusion

In this study, we successfully loaded diclofenac diethylammonium in liposomes with a high entrapment efficacy and a particle size below 100 nm. The properties of the developed liposomal cream conferred certain penetration advantages over a conventional formulation available on the market. Thereby, the speed of the liposome formulation through an artificial membrane was faster and the amount of active ingredient penetrated was significantly higher using *in vitro* assays. Whereas *ex vivo* assays showed that the active ingredient is able to penetrate the SC in greater quantity, providing an improvement compared to the conventional product. This finding could confer an advantage for clinical uses. Future work will further examine the in vivo capacity of the liposomal formulation to permeate.

## Ethical Approvals

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

## Conflicts of Interest

All authors are Industrias Biocontrolled, Grupo Leti S.A.V, employees. The authors declare no conflicts of interest regarding the publication of this paper.

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