Scientific Research Publishing

Journal of Biosciences and Medicines, 2024, 12, 316-327
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Acyclovir-Loaded Solid Lipid Nanoparticles: A Permeation and Penetrability Study

Anyoli Taly[1], Adriana Camino[1], Cirana Rodriguez[1], Evelyn Pena[2], Alfredo Inatti[1], Xenon Serrano[1*]

[1]Department of R&D, Nanotechnology Laboratory, Industrias Biocontrolled, Grupo Leti, S.A.V., Guarenas, Venezuela
[2]Department of Clinical Research, Industrias Biocontrolled, Grupo Leti, S.A.V., Guarenas, Venezuela
Email: *xenon.serrano@grupoleti.com

How to cite this paper: Taly, A., Camino, A., Rodriguez, C., Pena, E., Inatti, A. and Serrano, X. (2024) Acyclovir-Loaded Solid Lipid Nanoparticles: A Permeation and Penetrability Study. *Journal of Biosciences and Medicines*, **12**, 316-327.
https://doi.org/10.4236/jbm.2024.1210027

Received: September 4, 2024
Accepted: October 21, 2024
Published: October 24, 2024

Abstract

Herpes simplex virus type I is a cutaneous infection treated with acyclovir. The topical treatment has therapeutic challenges due to the deficient delivery of the drug through epithelial barriers. This results in an inadequate drug-virus interaction in the basal epidermis (virus replication site). For this reason, it is essential to generate drug carrier systems that overcome these limitations. In this study, we evaluated the permeation (through in vitro test Franz cells) and penetration (by *ex vivo* test Tape Stripping) of a topical formulation of acyclovir loaded in solid lipid nanoparticles and a conventional formulation (Aciclor®). The acyclovir solid lipid nanoparticles were prepared using hot homogenization and sonication methods. The results yielded a particle size of 85 ± 2 nm, a polydispersity index of 0.24 ± 0.01, a zeta potential of −16 ± 2 mV, and 94% ± 3% of encapsulated drug. The *in vitro* test revealed that the permeability of acyclovir solid lipid nanoparticles formulation was superior compared to reference formulation, with values of 1473.74 ± 30.14 μg/cm^2 for the solid lipid nanoparticles and 893.36 ± 38.09 μg/cm^2 for the reference formulation. The *ex vivo* test demonstrated that acyclovir solid lipid nanoparticles exhibited superior penetrability through the stratum corneum compared to the reference formulation, with total amounts of 3767 μg for the solid lipid nanoparticles and 2162 μg for the reference formulation. These findings seem promising in advancing new effective therapies against herpes generated by herpes simplex virus type I.

Keywords

Herpes, Acyclovir, Solid Lipid Nanoparticles, Franz Cells, Tape Stripping

1. Introduction

Herpesviruses cause chronic viral infections that remain in the body throughout

life and range from asymptomatic infections or mild cutaneous and mucocutaneous lesions to severe clinical manifestations such as encephalitis and aseptic meningitis, mainly in immunocompromised individuals and newborns. The herpes simplex virus type I (HSV-1) is highly contagious and mainly transmitted through oral-oral contact, which is typical and endemic worldwide. According to the World Health Organization, 3.7 billion people under the age of 50, or 67% of the world's population, have suffered from oral or genital HSV-1 infection. After primary infection of the skin or mucosa, it replicates locally in epithelial cells, causing their lysis and triggering an inflammatory response. Characteristic lesions of the infection consist of a thin-walled vesicle on an anti-inflammatory base. Primary HSV-1 infections generally occur during childhood as asymptomatic infections or as herpetic gingivostomatitis conditions and recurrent manifestations appear as orolabial lesions, called cold sores, fever blisters, or generally cold sores [1] [2]. Reactivation of the latent virus can be spontaneous but is more frequently associated with states of immunosuppression, emotional stress, hormonal changes, exposure to ultraviolet rays, or tissue damage. Once the virus is reactivated, it is transported anterogradely through the axon and mucocutaneous area, producing the lytic infection again [3].

Drugs to treat these infections are nucleoside analogs (antiviral agents) that phosphorylate and inhibit the synthesis of virus DNA when they enter the host cell. Acyclovir (ACV) is the prototype of this group of drugs and can be used orally, topically, or parenterally [3]. When HSV-1 viral infection is limited to the skin, topical administration of ACV is considered of special interest because higher levels of the active ingredient can be achieved in the basal epidermis (deepest layer), which is the area of maximum viral replication. However, it has been suggested that these topical formulations of ACV have low efficacy due to poor percutaneous penetration [4]. To achieve therapeutic concentrations in basal epidermal cells, treatment of epidermal viral infections requires repeated administration of high doses of ACV (400 - 1600 mg; 2 - 5 times a day for 5 to 10 days) [5]. Although ACV cream has some effect in healing skin lesions, it cannot prevent more of them even when applied in the prodromal stages [6]. Nanoscale topical drug delivery systems have attractive therapeutic potential according to their localized and sustained delivery capabilities. In addition, including a drug in nanoconstruct has demonstrated greater permeation and penetration capacities into epithelial barriers than conventional formulations [7]. Hence, solid lipid nanoparticles (SLN) offer several advantages, including safeguarding the active ingredient against chemical degradation and providing greater flexibility in drug release, among other benefits. SLN has been generated by simply exchanging the liquid lipid in emulsions for a solid lipid, meaning that SLN are solid at room temperature as well as body temperature. SLN are an alternative way to deliver lipophilic drugs with high efficiency and safety [8].

In this study, we evaluated the permeation and penetration capacity of an ACV SLN formulation and compared it with a conventional ACV formulation using in

vitro and *ex vivo* assays (Franz Cells and Tape Stripping, respectively).

2. Materials and Methods

Two topical formulations of ACV were used in these experiments: Test formulation (SLN 5% cream) and reference formulation (Aciclor® 5% cream, marketed by Leti Laboratories S.A.V., Venezuela).

2.1. Preparation of ACV Loaded SLN

The SLN were prepared by the hot homogenization method [9]. The aqueous phase consisted of emulsifying agents and parabens. The oil phase consisted of the fusion of the lipid and ACV at 80˚C Both phases were homogenized at 80˚C (Ultra Turrax, T25, DE). Finally, the suspension was ultrasonicated with an immersion diamond tip sonotrode (Vi-bra-Cell VCX750, Sonics, US) to form a nanosuspension of SLN upon cooling to 25˚C. Subsequently, the topical formulation of 5% ACV SLN was prepared, incorporating necessary excipients until the desired physicochemical characteristics were obtained.

2.2. Determination of Physicochemical Parameters

2.2.1. Particle Size and Zeta Potential Determination

The mean diameter of ACV SLN was determined using a dynamic light scattering instrument (Zetasizer Nano, model Zen 3600, Malvern, UK) at 25˚C with a fixed light angle of 90˚. As described by Sanabria *et al.* (2019) [10], measurements were performed with minor modifications. The samples were diluted (1:100) in distilled water. The particle size and polydispersity index (PDI) analysis data were further evaluated based on intensity distribution. The autocorrelation function was analyzed using Zetasizer 7.11 software. The zeta potential was also measured in the same instrument (Zetasizer Nano, model Zen 3600, Malvern, UK) using an immersion electrode (Dip Z cell) considering the Smoluchowski equation from the electrophoretic mobility of the SLN as a condition of the equipment. All data were generated at 25˚C in triplicate for statistical reasons.

2.2.2. Determination of the Percentage of Encapsulated ACV in the Nanosuspension

After preparing the nanodispersion and determining the physicochemical parameters, the SLN were separated by ultracentrifugation at 10,000 rpm, 4˚C for 45 minutes (Centrifuge 5430R, Eppendorf, DE). The ACV concentration present in the supernatant (non-encapsulated drug) was quantified using high-performance liquid chromatography equipment (HPLC) (Alliance, model 746, Waters, US), as described by Sanabria *et al.* (2019) [10] with minor modifications. The supernatant samples and standards were prepared in 0.01 N sodium hydroxide medium. The injection volume was 10 uL. A µBondapack C18 10 µm silica-based reversed-phase column was used (3.9 mm × 300 mm, Waters, USA). Elution was performed at 25˚C, with a mobile phase of 0.02 N acetic acid (100%), with a flow rate of 1.5 mL/min, 2500 psi. The UV detector wavelength

was set to 254 nm.

The percentage of encapsulated drug (pellet) was calculated by using the following equation:

$$\%\text{Encapsulated drug} = \frac{\text{total amount of ACV} - \text{Amount of unencapsulated ACV)}}{\left(\text{total amount of ACV}\right)} \times 100 \quad (1)$$

Total amount of ACV corresponds to what was initially added in the nanodispersion formula (included in the pellet and supernatant), and the amount of non-encapsulated ACV corresponds to the free drug found in the supernatant after centrifugation.

2.3. *In Vitro* Permeation Study (Franz Diffusion Cells)

For this study, diffusion assays were performed using the Franz cell method (Franz cells diffusion, model 58-001-430, Hanson Research, US) as described by Sanabria *et al.* (2019) [10], with slight modifications. A sample of 45 mg (equivalent to 2.25 mg of ACV) was weighted for the SLN and reference formulations in triplicate. It was then placed in the donor compartment of the Franz diffusion cell for further analysis. The acceptor volume compartment was 7 mL, and the diffusion membrane area was 1.77 cm^2. The receiving medium consisted of a previously sonicated and degassed phosphate buffer saline (pH: 7.4). A SIGMA (US) brand dialysis membrane with 12,500 Da of porosity was used, previously activated with washes of sodium sulfide and sulfuric acid solutions (procedure described in the supplier's product information [11]). A permeation profile was carried out at times 0, 0.5, 1, 3, and 24 hours at 37˚C with a stirring speed of 400 rpm. The ACV content was measured by HPLC in triplicate with the same conditions of section 2.2.2. Permeation profiles were plotted as the absolute (Q, μg/cm^2) and percentage (%) cumulative amount of ACV over 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, which is expressed by the following equation:

$$Kp = \frac{J}{C} \quad (2)$$

where *J* is the flow, and *C* is the amount of drug in the donor compartment [12].

2.4. *Ex Vivo* Penetrability Study (Tape Stripping Method)

This study was made on Saarbrücken support, according to Sanabria *et al.* (2019) [10], with modifications. Each skin disc of 25 mm in diameter and 1.3 mm in thickness [13], had 300 mg (equivalent to 15 mg of ACV) of a sample of each formulation (SLN and reference) in triplicate. After the 1-hour incubation period at 37˚C, the 20 strips (stratum corneum (SC)) and the remaining skin were collected and preserved in 4 mL of 0.01 N NaOH solution for 24 hours at 25˚C. The analysis of ACV content was determined by HPLC under the same conditions

described in section 2.2.2. The penetrability of ACV in the SC was represented in a histogram by the ratio of the mass of ACV (μg) as a function of each layer (strip) and the remaining skin. Tissue volume was calculated from the product of skin surface area (cm^2) and thickness (cm) [14].

2.5. Statistical Analysis

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.

3. Results

3.1. Physicochemical Parameters and Percentage of Encapsulated of ACV Loaded SLN

The dispersion was white, homogeneous and viscous. The data regarding the physicochemical characterization of the nanoconstructed product can be found in Table 1. Based on this, it was possible to develop a product with a particle size below 100 nm, monodisperse, with a high physical-chemical stability and a high percentage of encapsulated ACV.

Table 1. Physicochemical parameters and percentage encapsulated drug of ACV SLN dispersion.

Parameters	
Particle size (nm)	85 ± 2
PDI	0.24 ± 0.01
Zeta potential (mV)	−16 ± 2
Encapsulated drug (%)	94 ± 3

3.2. *In Vitro* Permeation Study (Franz Diffusion Cells)

Figure 1 shows the permeation profile of ACV SLN compared to the reference formulation. It was observed how the inclusion of ACV in SLN increases their permeation levels compared to the reference formulation. The cumulative amount of drug that penetrated through the membrane after 24 h in both formulations showed significant differences ($p < 0.05$), being greater in the SLN formulation (1473.74 ± 30.14 μg/cm^2) compared to the conventional formulation (893.36 ± 38.09 μg/cm^2). Moreover, these data were expressed in terms of percentage of ACV (Figure 2). The amount of drug present in the solid lipid nanoparticle (SLN) formulation after 24 hours was 93.17 ± 2.61%, significantly higher than the 68.26 ± 5.27% found in the reference formulation ($p < 0.05$). These results allowed us to calculate each formulation's maximum permeated flow and permeability coefficient (Table 2), resulting in a higher diffusion rate of SLN relative to the commercial formulation. This evidence suggests that the nano construction characteristics enhanced the permeation and increased the pass from the donor compartment to the recipient compared with the reference formulation.

Table 2. ACV permeated amount at 24 h, flow, and permeability coefficient.

Formulation	Permeated amount at 24 h (μg/cm²)	Flux (μg/cm² h)	Permeability coefficient (*Kp*) × 10⁻³ (cm/h)
Test (SLN)	1473.74 ± 30.14	58.895	1.8
Reference	893.36 ± 38.09	33.83	0.68

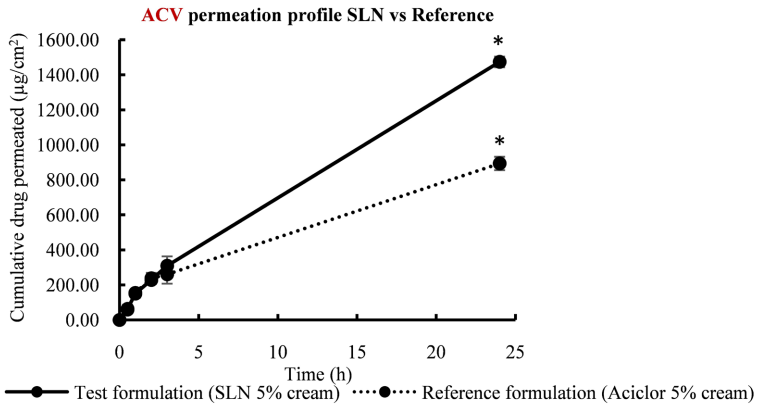

Figure 1. ACV permeation over the time of the SLN formulation vs. commercial formulation. (mean ± SD; n = 3; *p < 0.05).

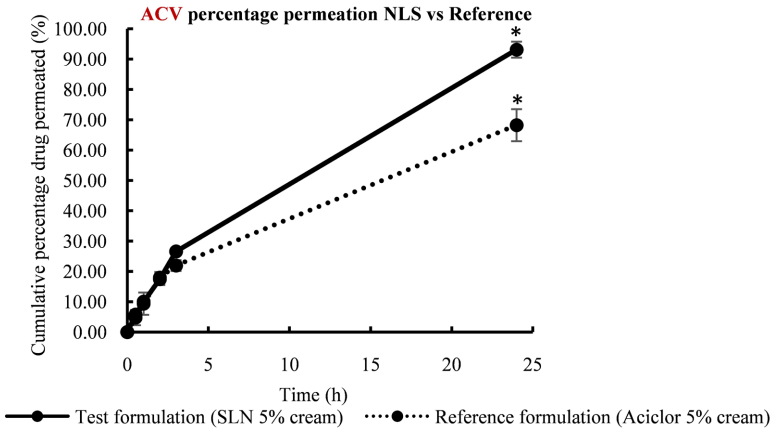

Figure 2. ACV percentage permeation over time of the SLN formulation vs. reference formulation (mean ± SD; n = 3; *p < 0.05).

3.3. *Ex Vivo* Penetrability Study (Tape Stripping Method)

Figure 3 compares the average mass of ACV present in the SC and the remaining skin between the SLN formulation and the conventional formulation. The presence of ACV in all the layers was observed for both formulations. However, a high amount of ACV is seen in each layer evaluated with the SLN formulation. The nanoconstructed product provides a more significant amount of drug (14% more) in the remaining skin than the reference formulation ($p < 0.05$). The total ACV values achieved in the 20 layers (SC) and the skin by the SLN formulation was

3767 µg (equivalent concentration of 5903 µg/mL), whereas the reference formulation was 2162 µg (equivalent concentration of 3678 µg/mL).

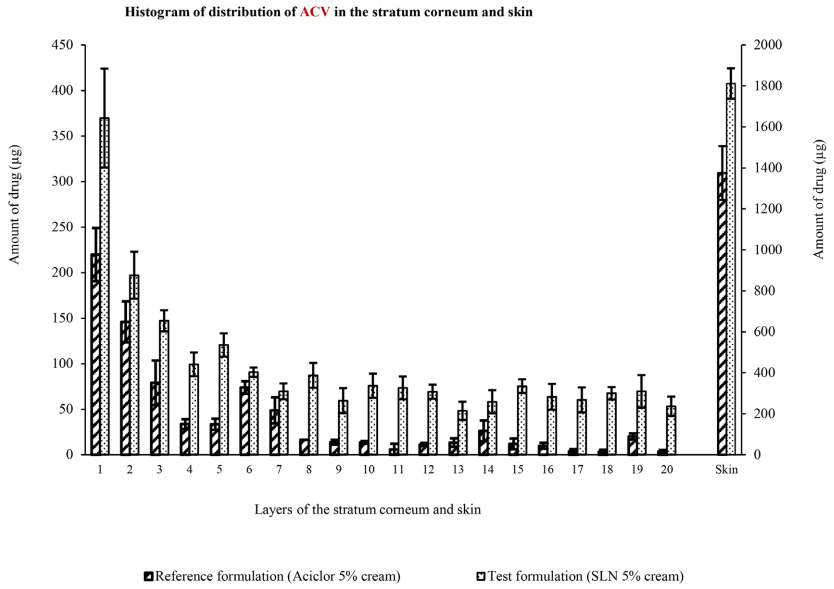

Figure 3. Comparison of the average amount of ACV present in the SC and skin between the test formulation vs. reference formulation. (mean ± SD; n = 3; $p < 0.05$).

4. Discussion

HSV-1 is a highly contagious, common, and endemic virus, affecting 67% of the world's population. The lesion consists of a thin-walled vesicle on a topical anti-inflammatory base, specifically orofacial [1] [2]. The first line of drug for treatment is ACV. However, it has been observed that the topical form exhibits low penetration through epithelial barriers, resulting in limited delivery to the basal epidermis, where the virus replicates [4]. This compromised penetration significantly impacts the drug's therapeutic efficacy [5].

In this work, an ACV formulation loaded in SLN with physicochemical and organoleptic characteristics was developed, which allowed a suitable topical product formulation. This product was compared with a reference formulation to assess pharmaceutical enhancement in terms of drug delivery using in vitro and ex vivo tests. It was demonstrated that the methodology used in this study to prepare the SLN effectively achieved an average particle size below 100 nm, with monomodal distribution, PDI < 0.5, and zeta potential < 0 mV. In this assay, it was employed two different shearing processes to minimize particle size: (1) high-speed homogenization, which resulted in sizes in the bimodal sub-micrometer range (specific data not presented), and (2) ultrasonication, recommended for achieving sizes in the nanometric range as per references [15], [16], and [17], aligning with the methodology applied in this study. The physicochemical parameters were a size of 85 ± 2 nm, PDI of 0.24 ± 0.01, and zeta potential of −16 ± 2 mV. We observed similar results concerning other ACV SLN topical formulations,

which generated particle sizes between 80 - 140 nm, PDI between 0.4 - 0.5, and zeta potential of −20 mV on average [18]. Other studies reported particle sizes between 200 - 400 nm (sub-micrometer range) and PDI between 0.4 - 0.8 using similar preparation protocols, concerning to homogenization without applying ultrasonication [19] [20] and using ultrasonication to reduce the particle size of nanoemulsion of acyclovir loaded in chitosan [1].

It is essential to consider not only the product's physicochemical characterization to ensure the nano construction preparation's efficacy but also the analysis of drug loading in the lipid matrix to provide evidence of its incorporation into the carrier. Our study demonstrated a favorable incorporation of ACV (94% ± 3%), despite its hydrophilic properties, within the encapsulated drug. The increase in the loading capacity requires that the drug accommodates inside the imperfections of the matrix, for which the lipid must solidify with a slightly ordered crystalline packing and present large intermolecular distances [9]. Likewise, the conditions of the drug-lipid-emulsifier relationship, homogenization speed, temperature, the solubility of the drug in the matrix, and the type of lipid significantly affect the encapsulation of the drug [15]. Against this evidence, similar results have been observed in other studies where ACV was incorporated into SLN, and the percentages of encapsulated drugs were between 30% - 80% [18]-[20] [21]. We observed an enhanced outcome compared to these studies, as our percentage of encapsulated drug represents an increase of 64% - 14%, respectively. These percentages were increased using different drug/lipid ratios [19]. Furthermore, varying the speed of homogenization from the lowest to the highest speed also contributes to an increase in the drug load. Regarding this evidence, we infer that our study's drug loading improvement is more related to the drug-lipid-emulsifier ratio. As mentioned above, the drug may have adjusted to the imperfections of the solid lipid, reducing its tendency to migrate to the aqueous phase. However, other rigorous tests are required to support these hypotheses.

The in vitro study showed an enhanced permeation of ACV SLN than the conventional formulation. Nevertheless, no differences were observed between the ACV SLN and the conventional product for the first 3 hours. This could be explained by a hypothesis suggesting that the drug incorporated in SLN remains more retained in the nanoconstructs and then diffuses into the cream matrix until reaching a stationary state before passing from the cream to the dissolution medium [22]. Based on this, we infer that this fact occurred in these first 3 hours. Furthermore, the increased ACV SLN diffusion after 3 hours may be attributed to its nanotechnological properties, which offer an advantage due to the particle size. The small size allows it to pass through the porous membrane more quickly, as evidenced during the last 21 hours. This phenomenon has been previously reported in the literature [23], which could contribute to our hypothesis. These trends have been observed in other studies that used biological membranes [18] [19], where the ACV SLN has a permeation pattern similar to the conventional formula during the first 3 hours and then differs significantly with higher SLN

permeation levels. This evidence supports our hypotheses, although it would be important to evaluate these diffusion phenomena in greater depth based on the interaction of the drug with the lipid matrix and the vehicle.

The *ex vivo* assay has shown an enhancement in drug delivery within nanocarriers through the skin. The percutaneous absorption of ACV SLN reaches higher levels in the remaining skin, where the basal stratum of the epidermis is located (target site) in which the lytic infection occurs. In addition, the concentration found in the remaining skin turned out to be above the drug concentration necessary to inhibit the growth of the virus in cell culture by 50% (IC_{50}: 0.02 to 13.5 μg/mL for HSV-1) [24]. The above would imply a potentially effective topical therapy. Nevertheless, other rigorous studies are required to compare the therapeutic efficacy of these products.

This similar phenomenon of percutaneous absorption of ACV SLN has been evidenced in other studies [19] where they evaluated the accumulated amount of ACV SLN *in vitro* tests on the skin of Sprague-Dawley rats, proving to be superior to conventional cream. In contrast, another discovery involved assessing liposomal ACV penetration in *ex vivo* tests conducted on mouse and human skin [25], demonstrating the great versatility of the liposome formulation compared to the conventional formulation. These studies indicate a similar behavior attributable to the nanostructure itself, despite the differences in chemical composition. In terms of ACV accumulation in pig ear skin, a previous study [1] reported that chitosan nanoparticles achieved an accumulation of 30% ACV in pig skin after 24 hours. In contrast, our study demonstrated that a 25% accumulation of ACV was attained in just 2 hours. This suggests that the lipid formulation represents a significant pharmaceutical improvement, enabling the delivery of a comparable amount of ACV to pig ear skin in a shorter testing timeframe than the chitosan formulation. Nonetheless, further rigorous testing is necessary to validate this hypothesis, including evaluations of longer *ex vivo* testing durations. These findings support the effectiveness of ACV delivery through the skin in a SLN topical formulation compared to reference product as observed in our study.

These drug penetration mechanisms have been described in the literature [13] [25]-[30]. The most effective factors for measuring transdermal drug delivery systems include the physicochemical characteristics and formulation type. The effectiveness of the treatment depends on the penetration of the drug through the target layers of the skin at adequate concentrations [13]. According to the literature, the movement of active substances through the SC generally occurs via intercellular between corneocytes, allowing more permeation and penetration [26]. Nonpolar drugs pass through this stratum via the intercellular route, while polar drugs pass via the transcellular [25] [27]. The intercellular spaces contain a well-structured lipid matrix, which represents a complex barrier that drug molecules must overcome to reach the skin's deeper layers (as in the stratum basale, target site). Therefore, cutaneous absorption depends on the physicochemical characteristics of the compounds and the specific application area [28]. For this reason, the use

of lipid nanocarriers is essential due to the physicochemical and pharmacological advantages that they offer. The lipid characteristics may promote a better interaction with the SC, helping the drug to be absorbed adequately (improved bioavailability). The particle size in the nanometric range facilitates close intercellular contact with the stratum corneum, ensuring effective delivery. Additionally, it enables sustained drug release, reduces systemic absorption, and provides an occlusive effect that enhances skin hydration and elasticity, thus facilitating enhanced drug penetration [29]. Based on the aforementioned, ACV, having a low penetration through the SC due to its hydrophilicity [1] [30] is highly enhanced by its inclusion in lipid nanocarriers. These improved physicochemical properties allow it to pass effectively through the different skin permeation pathways compared to the conventional product. This experimental study provides essential support for the future initiation of a Phase 1 clinical investigation aimed at evaluating the in vivo bioavailability of ACV SLN through these innovative cutaneous drug delivery systems.

5. Conclusion

In this study, it was obtained an ACV SLN formulation with particle size < 100 nm, PDI < 0.5, and an encapsulated drug > 90%. This physicochemical characteristic conferred an enhanced behavior drug permeation assay compared to the reference formulation (Aciclor 5% cream). The *ex vivo* assay showed an improved percutaneous absorption of ACV SLN through the SC compared to the conventional product. Furthermore, this novel formulation delivered a concentration of ACV to the target site (basal epidermis where the virus replicates) above the IC_{50} value for the HSV-1 virus. These findings provide new approaches for the effective treatment of topical herpes infections using formulations with nanotechnological properties.

Ethical Approvals

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

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Donalisio, M., Leone, F., Civra, A., Spagnolo, R., Ozer, O., Lembo, D., *et al.* (2018) Acyclovir-Loaded Chitosan Nanospheres from Nano-Emulsion Templating for the Topical Treatment of Herpesviruses Infections. *Pharmaceutics*, **10**, Article No. 46. https://doi.org/10.3390/pharmaceutics10020046

[2] World Health Organization (2023) Herpes Simplex Virus. https://www.who.int/news-room/fact-sheets/detail/herpes-simplex-virus

[3] Bascones, M.A. and Pousa, C.X. (2011) Herpesvirus. *Avances en Odontroestomatología*, **27**, 11-24. https://scielo.isciii.es/scielo.php?script=sci_arttext&pid=S0213-

1285201100010000 2#:~:text=El%20subgrupo%20alfa%2C%20conocido%20como,8%20o%20Sarcoma%20de%20Kaposi

[4] Chen, Y., Alberti, I. and Kalia, Y.N. (2016) Topical Iontophoretic Delivery of Ionizable, Biolabile Aciclovir Prodrugs: A Rational Approach to Improve Cutaneous Bioavailability. *European Journal of Pharmaceutics and Biopharmaceutics*, **99**, 103-113. https://doi.org/10.1016/j.ejpb.2015.12.002

[5] Saifi, Z., Rizwanullah, M., Mir, S.R. and Amin, S. (2020) Bilosomes Nanocarriers for Improved Oral Bioavailability of Acyclovir: A Complete Characterization through *in Vitro*, *Ex-Vivo* and *in Vivo* Assessment. *Journal of Drug Delivery Science and Technology*, **57**, Article ID: 101634. https://doi.org/10.1016/j.jddst.2020.101634

[6] Suárez, J.R., Rubio, A., Cuena, R., Azanza, J.R. and Honorato, J.M. (1984) Aciclovir. *Revista de Medicina de la Universidad de Navarra*, **30**, 125-129. https://revistas.unav.edu/index.php/revista-de-medicina/article/view/6370/5566

[7] Hussain, A., Altamimi, M.A., Afzal, O., Altamimi, A.S.A., Ramzan, M. and Khuroo, T. (2023) Mechanistic of Vesicular Ethosomes and Elastic Liposomes on Permeation Profiles of Acyclovir across Artificial Membrane, Human Cultured Epiderm, and Rat Skin: *In Vitro-Ex Vivo* Study. *Pharmaceutics*, **15**, Article No. 2189. https://doi.org/10.3390/pharmaceutics15092189

[8] Mateos, S.P. (2018) Sistemas de Liberación Modificada de Fármacos Antimicrobianos por Vía Parenteral. Ph.D. Thesis, Complutense University.

[9] Garzon, M., Hernandez, A., Vazquez, M., Villafuerte, L. and Garcia, B. (2008) Preparacion de Nanoparticulas Solidas Lipidicas (NLS) y de Acarreadores Lipidicos Nanoestructurados (NLC). *Revista Mexicana de Ciencias Farmacéuticas*, **39**, 50-66. https://www.redalyc.org/articulo.oa?id=57911113008

[10] Kinski, S., Antonieta, A.M., Nicolas, C. and Alfredo, I. (2019) Evaluation of the Permeation and Penetration of Two Formulations of Terbinafine Chlorhydrate Incorporated in Liposomes (Cream 1%) vs. a Conventional Formulation (Cream 1%), in an *in Vitro-Ex Vivo* Model. *Journal of Biosciences and Medicines*, **7**, 119-133. https://doi.org/10.4236/jbm.2019.78010

[11] SIGMA. Dialysis Tubing, Benzoylated, Product Information. D2272. https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/195/599/d2272pis.pdf

[12] Ghanbarzadeh, S. and Arami, S. (2013) Enhanced Transdermal Delivery of Diclofenac Sodium via Conventional Liposomes, Ethosomes, and Transfersomes. *BioMed Research International*, **2013**, Article ID: 616810. https://doi.org/10.1155/2013/616810

[13] Supe, S. and Takudage, P. (2020) Methods for Evaluating Penetration of Drug into the Skin: A Review. *Skin Research and Technology*, **27**, 299-308. https://doi.org/10.1111/srt.12968

[14] Parry, G.E., Dunn, P., Shah, V.P. and Pershing, L.K. (1992) Acyclovir Bioavailability in Human Skin. *Journal of Investigative Dermatology*, **98**, 856-863. https://doi.org/10.1111/1523-1747.ep12456948

[15] Bhatt, S., Sharma, J., Singh, M. and Saini, V. (2018) Solid Lipid Nanoparticles: A Promising Technology for Delivery of Poorly Water-Soluble Drugs. *ACTA Pharmaceutica Sciencia*, **56**, 27. https://doi.org/10.23893/1307-2080.aps.05616

[16] Kumar, R., Singh, A., Garg, N. and Siril, P.F. (2018) Solid Lipid Nanoparticles for the Controlled Delivery of Poorly Water Soluble Non-Steroidal Anti-Inflammatory Drugs. *Ultrasonics Sonochemistry*, **40**, 686-696. https://doi.org/10.1016/j.ultsonch.2017.08.018

[17] Khare, A., Singh, I., Pawar, P. and Grover, K. (2016) Design and Evaluation of Voriconazole Loaded Solid Lipid Nanoparticles for Ophthalmic Application. *Journal of Drug Delivery*, **2016**, Article ID: 6590361. https://doi.org/10.1155/2016/6590361

[18] Gide, P., Gidwani, S. and Kothule, K. (2013) Enhancement of Transdermal Penetration and Bioavailability of Poorly Soluble Acyclovir Using Solid Lipid Nanoparticles Incorporated in Gel Cream. *Indian Journal of Pharmaceutical Sciences*, **75**, 138-142. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3757850/

[19] El Assal, M. (2017) Acyclovir Loaded Solid Lipid Nanoparticle Based Cream: A Novel Drug Delivery System. *International Journal of Drug Delivery Technology*, **7**, 52-62. https://doi.org/10.25258/ijddt.v7i1.8917

[20] Jain, S., Mistry, M.A. and Swarnakar, N.K. (2011) Enhanced Dermal Delivery of Acyclovir Using Solid Lipid Nanoparticles. *Drug Delivery and Translational Research*, **1**, 395-406. https://doi.org/10.1007/s13346-011-0036-0

[21] Parthiban, R., *et al.* (2020) Design and Evaluation of Acyclovir-Loaded Solid Lipid Nanoparticles for Sustained Release. *Drug Invention Today*, **14**, 108-111. https://www.researchgate.net/publication/340396996_Design_and_evaluation_of_acyclovir-loaded_solid_lipid_nanoparticles_for_sustained_release

[22] Tiyaboonchai, W., Tungpradit, W. and Plianbangchang, P. (2007) Formulation and Characterization of Curcuminoids Loaded Solid Lipid Nanoparticles. *International Journal of Pharmaceutics*, **337**, 299-306. https://doi.org/10.1016/j.ijpharm.2006.12.043

[23] Pardeike, J., Hommoss, A. and Müller, R.H. (2009) Lipid Nanoparticles (SLN, NLC) in Cosmetic and Pharmaceutical Dermal Products. *International Journal of Pharmaceutics*, **366**, 170-184. https://doi.org/10.1016/j.ijpharm.2008.10.003

[24] Acyclovir Ointment Prescribing Information. https://www.drugs.com/pro/acyclovir-ointment.html

[25] Gutierrez, R. (2011) Estudios de Difusión a Través de Piel de Formulaciones Liposómicas de Aciclovir. Ph.D. Thesis, Complutense University of Madrid.

[26] Mehnert, W. (2001) Solid Lipid Nanoparticles Production, Characterization and Applications. *Advanced Drug Delivery Reviews*, **47**, 165-196. https://doi.org/10.1016/s0169-409x(01)00105-3

[27] Taveira, S.F. (2009) Solid Lipid Nanoparticles (SLN) as Drug Carriers for the Topical Treatment of Skin Cancer. Ph.D. Thesis, University of São Paulo.

[28] Schaferkorting, M., Mehnert, W. and Korting, H. (2007) Lipid Nanoparticles for Improved Topical Application of Drugs for Skin Diseases. *Advanced Drug Delivery Reviews*, **59**, 427-443. https://doi.org/10.1016/j.addr.2007.04.006

[29] Samimi, S., Maghsoudnia, N., Eftekhari, R.B. and Dorkoosh, F. (2019) Lipid-Based Nanoparticles for Drug Delivery Systems. In: Mohapatra, S.S., *et al.*, Eds., *Characterization and Biology of Nanomaterials for Drug Delivery*, Elsevier, 47-76. https://doi.org/10.1016/b978-0-12-814031-4.00003-9

[30] Sánchez Belloso, A. (2011) Use of Nanoparticles in Acyclovir Formulations. *Complutense Magazine of Veterinary Sciences*, **11**, 126-131. https://revistas.ucm.es/index.php/RCCV/article/download/55359/50373