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Engineering and Characterization of Acyclovir-Loaded Nano-Niosomes for Enhanced Antiviral Drug Delivery

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Abstract

Acyclovir is a widely used antiviral drug for the treatment of Herpes Simplex Virus (HSV) infections; however, its limited aqueous solubility and poor bioavailability may restrict its therapeutic effectiveness. The present study aimed to develop and evaluate acyclovir-loaded nanoniosomes as a nano-based vesicular delivery system to improve drug encapsulation, stability, and controlled release properties. Acyclovir-loaded nanoniosomes were prepared using the thin-film hydration method, and five formulations were developed by varying the Span 60-to-cholesterol molar ratio (4:1, 3:1, 2:1, 1.5:1, and 1:1). The prepared formulations were characterized regarding particle size, Polydispersity Index (PDI), zeta potential, Entrapment Efficiency (EE%), drug Loading Capacity (LC%), in vitro drug release, and pH stability. The results demonstrated successful formation of nanosized vesicular systems in all formulations. Among the prepared formulations, F3 with a Span 60 molar ratio of 2:1 showed the most favorable physicochemical characteristics, including a particle size of 185.6±0.4 nm and a PDI value of 0.24± 0.03. The optimized formulation exhibited a zeta potential of −31.4±0.1 mV, indicating acceptable colloidal stability. Furthermore, F3 demonstrated high entrapment efficiency (79.6±0.1%) and suitable drug loading capacity. The in vitro release study showed a sustained release profile, with approximately 78.4% of acyclovir released after 24 h. The formulation maintained relatively constant pH values during storage, confirming its acceptable stability. Overall, the developed acyclovir-loaded nanoniosomal system provided nanoscale vesicles with appropriate stability, efficient drug incorporation, and controlled release behavior. The optimized formulation may represent a promising nano-based carrier for improving the topical delivery of acyclovir.

Keywords: Acyclovir, Nanoniosomes, Span 60, Cholesterol, Nanovesicles, Controlled drug release, Topical delivery.

1 | Introduction

Herpes Simplex Virus (HSV) infections are among the most common viral diseases affecting humans worldwide [1–3]. HSV exists in two main types, including Herpes Simplex Virus Type 1 (HSV-1) and Herpes

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Simplex Virus Type 2 (HSV-2), which are responsible for a variety of clinical manifestations ranging from mild mucocutaneous lesions to severe infections in immunocompromised patients [2]. HSV-1 is mainly associated with oral and facial infections, while HSV-2 is commonly related to genital infections [2–4]. Due to the ability of HSV to establish lifelong latency in host nerve cells and reactivate periodically, effective therapeutic strategies are required to reduce viral replication, accelerate lesion healing, and minimize the recurrence rate. Although several antiviral agents have been developed, improving their therapeutic performance remains an important challenge in pharmaceutical research [5]. Acyclovir is a widely used antiviral drug belonging to the nucleoside analogue class and is considered one of the first-line treatments for HSV infections. The antiviral activity of acyclovir is based on selective phosphorylation by viral thymidine kinase, followed by inhibition of viral DNA polymerase, resulting in suppression of viral DNA synthesis [4–7]. Despite its high selectivity and clinical effectiveness, acyclovir has several pharmaceutical limitations that restrict its optimal therapeutic application. The drug possesses poor aqueous solubility, limited permeability through biological membranes, and relatively low bioavailability [5], [8–10]. Furthermore, conventional topical formulations of acyclovir often require frequent administration due to rapid drug removal from the application site, which may decrease patient compliance and reduce therapeutic efficiency [9]. Therefore, developing advanced drug delivery systems capable of improving acyclovir stability, prolonging residence time, and enhancing drug penetration is highly desirable. Nanotechnology-based drug delivery systems have attracted considerable attention as promising approaches for overcoming the limitations associated with conventional formulations [10–12]. Nanocarriers can improve drug solubility, protect active pharmaceutical ingredients from degradation, control drug release, and enhance the accumulation of therapeutic agents at the target site [13]. Among various nanocarriers, niosomes have emerged as effective vesicular systems for the delivery of both hydrophilic and hydrophobic compounds. Niosomes are non-ionic surfactant-based vesicles consisting of non-ionic surfactants and cholesterol, which form a bilayer structure capable of encapsulating active molecules [10–14].

Compared with liposomes, niosomes provide several advantages, including higher chemical stability, lower cost, improved storage properties, and easier large-scale production. Nanoniosomes, which represent nanosized niosomal vesicles, have recently gained increasing interest due to their enhanced surface area, improved cellular interaction, and superior drug delivery potential [15]. The small particle size of nanoniosomes can promote better penetration through biological barriers and provide controlled release characteristics. Additionally, the incorporation of cholesterol into the niosomal membrane improves vesicle rigidity and reduces drug leakage, resulting in improved formulation stability [16]. These properties make nanoniosomes attractive candidates for topical delivery of antiviral drugs such as acyclovir, where prolonged contact with the skin and enhanced local drug concentration are essential for achieving optimal therapeutic effects [17–19]. Topical delivery of acyclovir using nanoniosomal systems may provide several advantages over conventional formulations, including increased drug retention at the site of infection, sustained release, enhanced penetration into infected tissues, and improved antiviral activity. Furthermore, the development of a nanoniosomal gel formulation may combine the benefits of nanoscale vesicular carriers with the convenience and patient acceptability of topical dosage forms [18–20].

Therefore, the aim of the present study is to formulate, optimize, and characterize acyclovir-loaded nanoniosomes and evaluate their potential as an improved topical drug delivery system against HSV infection. The prepared formulations will be investigated regarding particle size, Polydispersity Index (PDI), zeta potential, encapsulation efficiency, morphology, in vitro drug release behavior, and stability. The developed nanoniosomal system is expected to enhance the pharmaceutical performance of acyclovir and provide a more efficient approach for topical antiviral therapy.

As illustrated in *Fig. 1*, niosomal vesicles interact with the stratum corneum and facilitate deeper transdermal drug delivery.

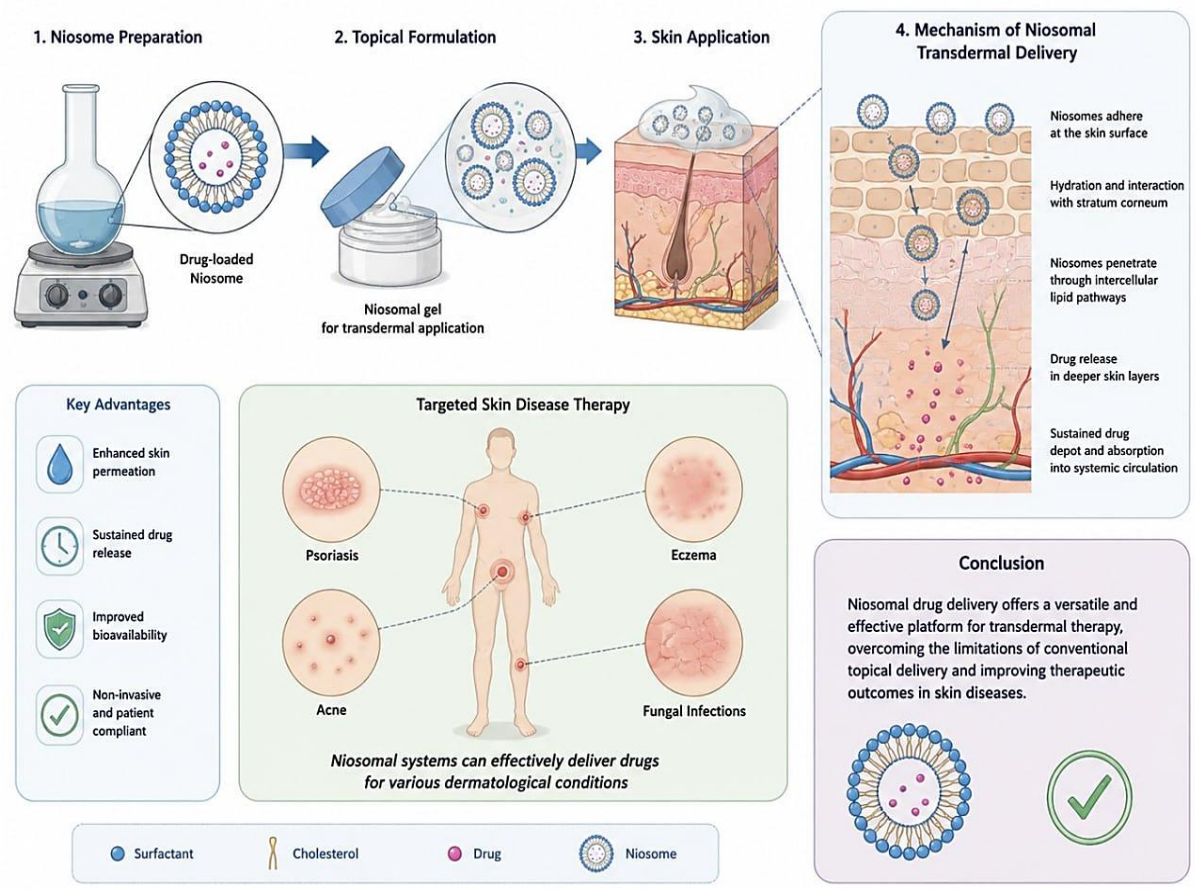

Fig. 1. Schematic overview of niosome preparation, topical formulation, and transdermal drug delivery pathways.

2 | Materials and Methods

2.1 | Materials

Acyclovir was used as the model antiviral drug. Span 60 (sorbitan monostearate) and cholesterol were employed for the preparation of nanoniosomes. Chloroform and methanol were used as organic solvents. Phosphate-buffered saline (PBS, pH 7.4) was used as hydration and release medium. Distilled water was used throughout the experiments.

2.2 | Preparation of Acyclovir-Loaded Nanoniosomes

Nanoniosomes were prepared by the thin-film hydration method. Span 60 and cholesterol were mixed at a molar ratio of 1:1. Briefly, 200 mg of Span 60 and 100 mg of cholesterol were dissolved in 10 mL of chloroform mixture (2:1, v/v) in a 100-mL round-bottom flask. The organic solvent was removed using a rotary evaporator at 60°C and 100 rpm under reduced pressure for 30 min to obtain a thin lipid film on the inner wall of the flask. The resulting film was kept under vacuum overnight to ensure complete removal of residual solvents. Hydration was carried out using 10 mL phosphate-buffered saline (PBS, pH 7.4) containing 50 mg acyclovir. The hydration process was performed at 60°C for 30 min under gentle rotation. The resulting multilamellar vesicles were reduced to nanosized vesicles using probe sonication for 5 min in an ice bath. The final nanoniosomal dispersion was stored at 4°C for further characterization.

2.3 | Particle Size Analysis

The mean particle size and PDI of acyclovir-loaded nanoniosomes were determined by Dynamic Light Scattering (DLS). Prior to analysis, samples were diluted with deionized water (1:100) and analyzed at 25°C. Measurements were performed in triplicate, and the results were expressed as mean ± standard deviation. The average particle size and PDI were recorded to evaluate the size distribution and homogeneity of the nanoniosomal formulations.

2.4 | Zeta Potential Measurement

The zeta potential of the prepared acyclovir-loaded nanoniosomes was determined using a zeta potential analyzer after appropriate dilution with deionized water. Measurements were carried out at 25°C to evaluate the surface charge and physical stability of the nanoniosomal formulations. All samples were analyzed in triplicate, and the results were expressed as mean ± standard deviation.

2.5 | Entrapment Efficiency

The Entrapment Efficiency (EE%) of acyclovir-loaded nanoniosomes was determined by separating the unentrapped drug from the vesicular dispersion using centrifugation. Briefly, the formulation was centrifuged at 15000 rpm for 30 min, and the amount of free acyclovir in the supernatant was quantified spectrophotometrically at 252 nm. The entrapment efficiency was calculated using the following *Eq. (1)* [20]:

$$\text{EE}\ (\%) = \frac{(\text{Total drug} - \text{Free drug})}{\text{Total drug}} \times 100. \quad \textbf{(1)}$$

All measurements were performed in triplicate, and the results were expressed as mean ± standard deviation.

2.6 | In Vitro Drug Release Study

The in vitro release of acyclovir from the prepared nanoniosomes was evaluated using the dialysis bag diffusion method. Briefly, 1 mL of the nanoniosomal dispersion was placed in a dialysis membrane (MWCO 12–14 kDa) and immersed in 100 mL phosphate-buffered saline (PBS, pH 7.4). The release medium was maintained at 37 °C under continuous stirring at 100 rpm. At predetermined time intervals, aliquots were withdrawn and replaced with an equal volume of fresh medium to maintain sink conditions. The amount of released acyclovir was determined using UV–Visible spectrophotometry at 252 nm. All experiments were performed in triplicate, and the cumulative percentage of drug release was calculated and plotted as a function of time.

2.7 | Drug Loading Capacity

The drug Loading Capacity (LC%) of the prepared acyclovir-loaded nanoniosomes was determined to evaluate the amount of acyclovir incorporated within the nanoniosomal formulation. A known amount of freeze-dried nanoniosomal formulation (or a measured volume of nanoniosomal dispersion) was dissolved in a suitable solvent to disrupt the vesicular structure and release the entrapped drug.

The amount of acyclovir present in the formulation was quantified using UV–Visible spectrophotometry at 252 nm. The drug loading capacity, was calculated using the following *Eq. (2)* [20]:

$$\text{LC}\ (\%) = \frac{\text{Amount of entrapped drug}}{\text{Total amount of nanoniosomal formulation}} \times 100. \quad \textbf{(2)}$$

All measurements were performed in triplicate, and the results were expressed as mean ± standard deviation.

2.8|pH Stability Study

The pH stability of the optimized acyclovir-loaded nanoniosomal formulation was evaluated during storage. The pH values of the formulations were measured using a calibrated digital pH meter at predetermined time intervals. The samples were stored under different conditions (4°C and 25°C), and pH changes were monitored over a period of three months. The stability of the formulation was evaluated by comparing the initial pH value with the values obtained during storage. All measurements were performed in triplicate, and the results were expressed as mean ± standard deviation.

2.9|Stability Study

The stability of the optimized acyclovir-loaded nanoniosomal formulation was evaluated under different storage conditions. Samples were stored at refrigerated (4°C) and room temperature (25°C) conditions for a period of three months. At predetermined time intervals, the formulations were evaluated for changes in particle size, PDI, zeta potential, and entrapment efficiency. Any physical changes, such as aggregation, precipitation, or phase separation, were also visually monitored. All measurements were performed in triplicate, and the results were expressed as mean ± standard deviation.

3|Results & Discussion

3.1|Particle Size and Polydispersity Index

The particle size and size distribution of acyclovir-loaded nanoniosomes were evaluated using DLS. The optimized formulation showed a nanoscale particle size with an average diameter of 185.6 ±0.4 nm. The obtained particle size indicated successful formation of nanoniosomal vesicles. The relatively small vesicle size may provide a higher surface area, improved contact with biological membranes, and enhanced potential for topical drug delivery. The PDI of the optimized formulation was found to be 0.24±0.03, indicating a narrow particle size distribution and acceptable formulation homogeneity.

3.2|Zeta Potential Analysis

The zeta potential of all acyclovir-loaded nanoniosomal formulations was measured to evaluate the surface charge and physical stability of the vesicular systems. The obtained results are summarized in *Table 1*.

Table 1. Zeta potential values of acyclovir-loaded nanoniosomal formulations.

Formulation	Span 60: Cholesterol Ratio	Zeta Potential (mV)
F1	4:1	-21.3±0.7
F2	3:1	-26.8±0.9
F3	2:1	-31.4±0.1
F4	1.5:1	-34.2±0.8
F5	1:1	-28.6±0.3

As shown in *Table 1*, all formulations exhibited negative zeta potential values, indicating the formation of negatively charged vesicles. The negative surface charge contributes to electrostatic repulsion between vesicles, thereby reducing aggregation and improving the physical stability of the nanoniosomal dispersions. The zeta potential values gradually increased in magnitude from F1 to F4 as the cholesterol content increased. This observation suggests that cholesterol plays an important role in improving membrane organization and stabilizing the vesicular structure. Formulations F3 and F4 exhibited zeta potential values greater than −30 mV, which are generally considered indicative of good colloidal stability. Among the investigated formulations, F4 showed the highest absolute zeta potential value (−34.2±0.8 mV), suggesting excellent electrostatic stabilization. However, despite its higher zeta potential, F4 exhibited a larger particle size and a

higher PDI compared with F3. Therefore, zeta potential alone could not be considered the sole criterion for formulation optimization. Formulation F3 demonstrated a favorable balance between particle size, size distribution, and surface charge, exhibiting a particle size of 185.6±0.4 nm, a PDI of 0.24±0.03, and a zeta potential of −31.4±0.1 mV. The combination of these characteristics indicates the formation of a stable and homogeneous nanosystem. Interestingly, a further increase in cholesterol concentration (F5) resulted in a reduction in the absolute zeta potential value. This behavior may be attributed to excessive cholesterol incorporation into the bilayer structure, which can alter vesicle organization and reduce the effective surface charge of the nanoniosomes. The results demonstrated that cholesterol concentration significantly influenced the surface properties of the nanoniosomal formulations. Based on the combined evaluation of particle size, PDI, and zeta potential, formulation F3 (Span 60 ratio of 2:1) was selected as the optimized formulation for further characterization studies.

3.3 | Entrapment Efficiency

The entrapment efficiency of acyclovir-loaded nanoniosomal formulations was evaluated to determine the ability of the vesicular systems to encapsulate and retain the drug within the bilayer structure. The results are presented in *Fig. 2*.

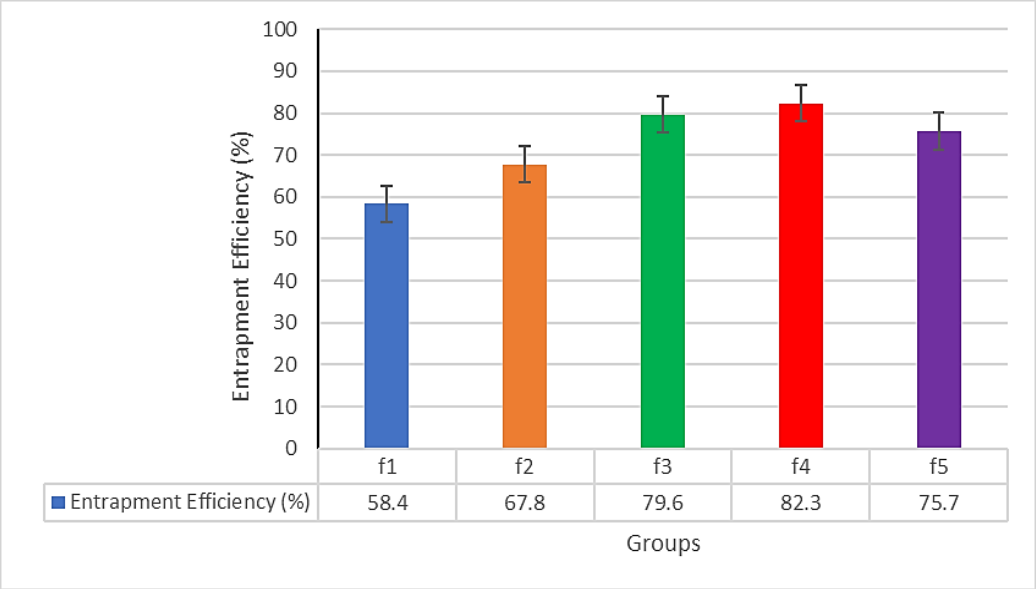

Fig. 2. Entrapment efficiency of acyclovir-loaded nanoniosomal formulations.

As shown in *Fig. 2*, the entrapment efficiency of the formulations increased progressively from F1 to F4 with increasing cholesterol concentration. Cholesterol is known to enhance the rigidity and integrity of the vesicular bilayer, thereby reducing drug leakage and improving drug retention within the nanoniosomes. Formulation F1 exhibited the lowest entrapment efficiency (58.4±0.3%), which may be attributed to insufficient membrane stabilization and increased permeability of the vesicular bilayer. Increasing the cholesterol content from F1 to F4 resulted in a significant improvement in drug encapsulation efficiency. Among the investigated formulations, F4 showed the highest entrapment efficiency (82.3±0.4%). This finding suggests that the cholesterol content in F4 provided an optimal membrane environment for retaining acyclovir within the vesicular structure. However, further increasing the cholesterol concentration to a 1:1 molar ratio (F5) resulted in a decrease in entrapment efficiency. This reduction may be attributed to excessive cholesterol incorporation within the bilayer, which can compete with drug molecules for space within the vesicular membrane and reduce the available volume for drug entrapment. Although F4 exhibited the highest EE%, formulation F3 demonstrated a comparable entrapment efficiency (79.6±0.1%) while maintaining the smallest particle size, lowest PDI, and satisfactory zeta potential. Therefore, considering all physicochemical characteristics collectively, F3 was selected as the optimized formulation for subsequent studies. The obtained

results indicate that cholesterol concentration plays a crucial role in determining the drug encapsulation capacity of nanoniosomes, and an appropriate balance between membrane rigidity and vesicle organization is essential for achieving efficient drug loading.

3.4 | In Vitro Drug Release Study

The in vitro release behavior of acyclovir from different nanoniosomal formulations was investigated using the dialysis bag diffusion method in phosphate-buffered saline (PBS, pH=7.4) at 37°C. The cumulative percentage of drug release was determined at predetermined time intervals, and the results are presented in *Fig. 3*.

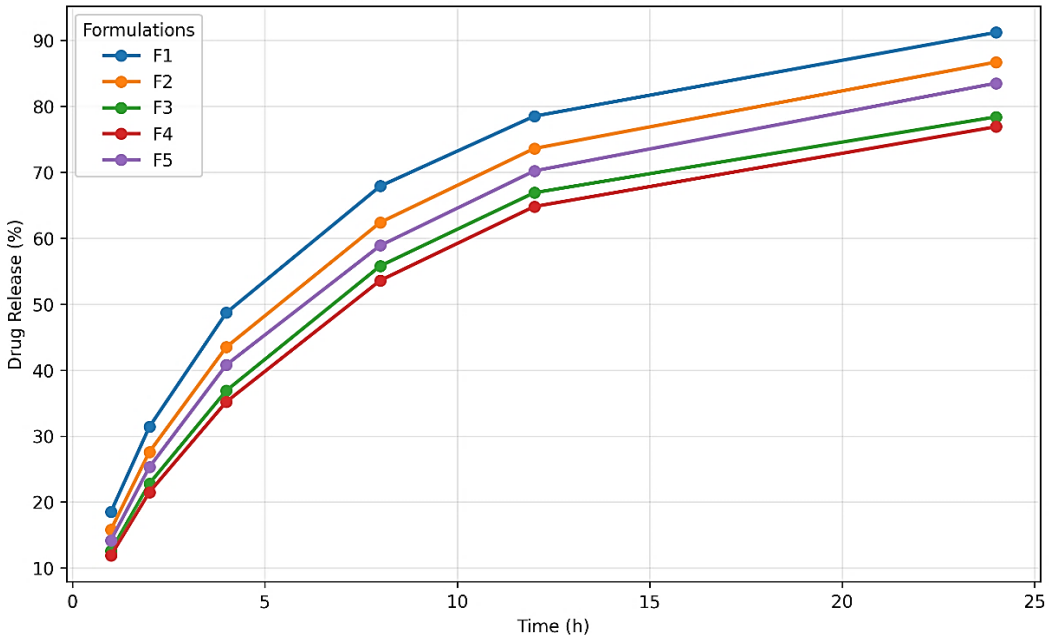

Fig. 3. Cumulative release percentage of acyclovir from nanoniosomal formulations.

The obtained results demonstrated a sustained release behavior of acyclovir from all nanoniosomal formulations compared with rapid drug diffusion from conventional drug solutions. The release rate was influenced by the composition of the vesicular bilayer, particularly the cholesterol concentration. Formulation F1 showed the highest drug release after 24 h (91.2±0.1%), which may be related to the lower cholesterol content and higher membrane fluidity, resulting in faster drug diffusion through the vesicular membrane. Increasing cholesterol concentration resulted in a gradual reduction in the release rate. Formulation F3 showed a controlled release profile with approximately 78.4±0.2% drug release after 24 h. The slower release from F3 may be attributed to improved bilayer organization and increased membrane rigidity caused by cholesterol incorporation. Although F4 demonstrated slightly slower release compared with F3, its larger particle size and higher PDI suggested lower overall formulation homogeneity. Therefore, F3 was considered the optimized formulation due to its balanced physicochemical properties and sustained drug release behavior. The controlled release pattern observed for the optimized nanoniosomal formulation may improve drug residence time at the application site and potentially enhance the therapeutic efficiency of topical acyclovir delivery.

3.5 | Drug Loading Capacity

The drug loading capacity of acyclovir-loaded nanoniosomal formulations was determined to evaluate the amount of acyclovir incorporated within the nanoniosomal system. The obtained results are presented in *Fig. 4*.

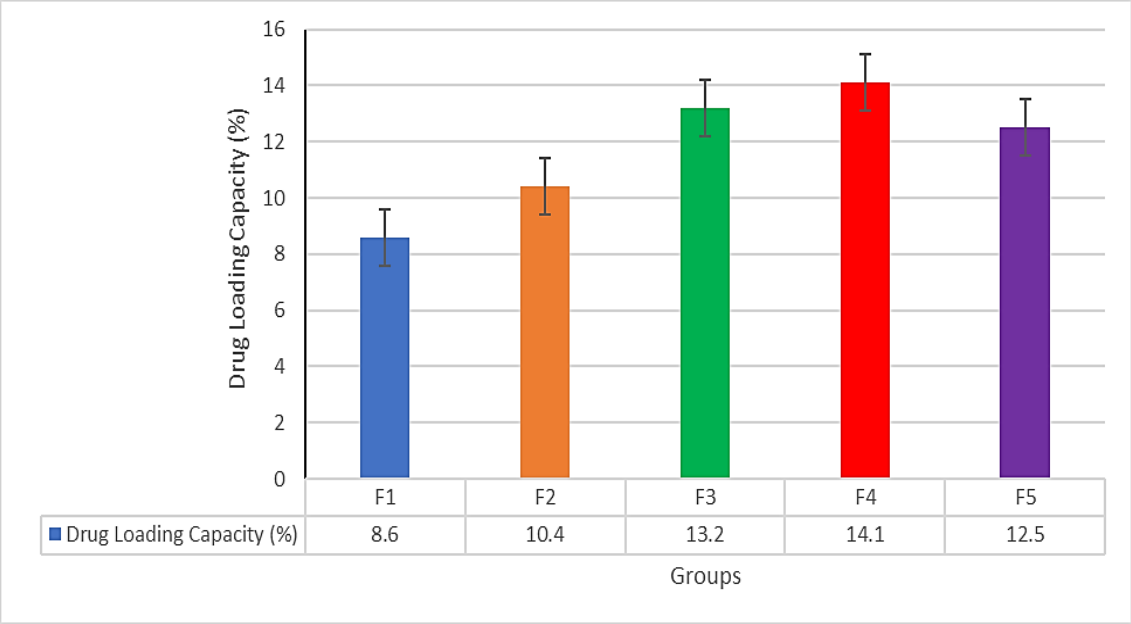

Fig. 4. Drug loading capacity of acyclovir-loaded nanoniosomal formulations.

The obtained results showed that the drug loading capacity, increased with increasing cholesterol concentration up to formulation F4. The improvement in LC% may be attributed to the enhanced organization and rigidity of the nanoniosomal bilayer caused by cholesterol incorporation, which improves the ability of the vesicles to retain acyclovir. The lowest LC% was observed in formulation F1 (8.6±0.5%), which may be related to the lower cholesterol content and reduced vesicle stability, resulting in lower drug incorporation capacity. Formulation F4 exhibited the highest drug loading capacity (14.1±0.8%), suggesting a greater ability of the vesicular structure to accommodate acyclovir. However, excessive cholesterol concentration in F5 resulted in a reduction in LC%, which may be due to decreased available space within the bilayer structure and alteration of vesicle composition. Although F4 showed the highest LC%, formulation F3 demonstrated a more balanced physicochemical profile with a smaller particle size, lower PDI, acceptable zeta potential, and high drug encapsulation efficiency. Therefore, F3 was selected as the optimized nanoniosomal formulation. The results indicate that an appropriate Span 60-to-cholesterol ratio is essential for achieving optimal drug loading and maintaining stable nanosized vesicles.

3.6 | pH Stability Study

The pH stability of acyclovir-loaded nanoniosomal formulations was evaluated during storage to investigate possible changes in formulation stability. The pH values of the prepared formulations were measured at predetermined time intervals under different storage conditions. The obtained results are presented in *Table 2*.

Table 2. pH changes of acyclovir-loaded nanoniosomal formulations during storage.

Formulation	Initial pH	After 1 Month	After 3 Months
F1	6.42±0.06	6.35±0.08	6.28±0.07
F2	6.48±0.05	6.43±0.06	6.36±0.08
F3	6.51±0.04	6.48±0.05	6.44±0.06
F4	6.54±0.05	6.49±0.06	6.43±0.07
F5	6.47±0.06	6.38±0.07	6.30±0.09

The obtained results showed that all nanoniosomal formulations maintained relatively constant pH values during the storage period, indicating acceptable physicochemical stability of the prepared vesicular systems. A slight decrease in pH was observed in all formulations after three months of storage, which may be related to minor chemical changes or drug–vesicle interactions during storage. However, the observed variations were not significant. The optimized formulation (F3) showed minimal pH variation, changing from 6.51 ± 0.04 to 6.44 ± 0.06 after three months. The maintained pH value within a skin-compatible range suggests that the formulation is suitable for topical application. The low changes in pH values confirm the stability of the nanoniosomal system and support the suitability of the optimized formulation for acyclovir topical delivery.

4|Conclusion

In the present study, acyclovir-loaded nanoniosomes were successfully developed as a nano-based vesicular drug delivery system using the thin-film hydration method. Different formulations were prepared by changing the Span 60-to-cholesterol molar ratio in order to investigate the influence of vesicle composition on the physicochemical properties and performance of the nanoniosomal system. The obtained results demonstrated that the incorporation of cholesterol had a significant effect on the characteristics of the prepared formulations. All prepared formulations showed nanoscale particle sizes, confirming the successful formation of nanoniosomal vesicles. Among the investigated formulations, F3 with a Span 60 molar ratio of 2:1 exhibited the most favorable particle characteristics, including the smallest particle size (185.6±0.4 nm) and the lowest PDI (0.24±0.03), indicating the formation of a homogeneous nanosized system with a relatively narrow size distribution. The surface charge analysis showed that all formulations possessed negative zeta potential values, which contributed to the stability of the vesicular dispersions by providing electrostatic repulsion between particles. The optimized formulation (F3) showed a zeta potential of −31.4±0.1 mV, suggesting acceptable colloidal stability and reduced tendency toward particle aggregation during storage. The drug encapsulation studies revealed that increasing cholesterol concentration improved acyclovir entrapment efficiency by enhancing bilayer organization and reducing drug leakage. Although formulation F4 demonstrated the highest entrapment efficiency, F3 provided a better overall balance between drug incorporation, particle size, size uniformity, and surface charge. The optimized formulation showed high entrapment efficiency (79.6 ±0.1%) and suitable drug loading capacity, confirming the ability of the nanoniosomal system to effectively incorporate acyclovir. The in vitro release study demonstrated that the nanoniosomal formulation provided a controlled and sustained release pattern compared with rapid drug diffusion from conventional formulations. The optimized formulation showed a prolonged release profile, which may improve drug residence time at the application site and enhance the efficiency of topical acyclovir delivery. Furthermore, the stability evaluation showed minimal changes in pH values during storage, indicating acceptable physicochemical stability of the prepared nanoniosomes. The maintained pH within a skin-compatible range supports the potential suitability of the formulation for topical administration. The developed acyclovir-loaded nanoniosomal system successfully combined nanoscale size, good physical stability, high drug encapsulation efficiency, and controlled drug release characteristics. The optimized F3 formulation (Span 60 ratio of 2:1) demonstrated the most balanced physicochemical properties and could be considered a promising nano-based carrier for improving the topical delivery of acyclovir.

Authors' Contributions

The author was responsible for all stages of the research and manuscript preparation and approved the final version.

Data Availability

All data are included in the text.

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Conflict of Interest

There are no competing interests to declare.

Consent for Publication

The author confirms consent for the publication of this work

Ethics Approval and Consent to Participate

This article does not contain any studies with human participants performed by the author.

References

[1] Kumar, D., Sahani, V., & Patil, S. (2025). Acyclovir cream: A compressive review of development, characterization and therapeutic efficacy. *Journal for Research in Applied Sciences and Biotechnology Учредители: Stallion Publication*, *4*(1), 27–37. https://doi.org/10.55544/jrasb.4.1.3

[2] Aboelezz, A., Kharouba, M., & Mahmoud, S. H. (2025). Acyclovir dosing strategies in herpes encephalitis: A retrospective charts review. *Journal of Clinical Neuroscience*, *136*, 111230. https://doi.org/10.1016/j.jocn.2025.111230

[3] Whitley, R. J., & Gnann Jr, J. W. (1992). Acyclovir: A decade later. *New England Journal of Medicine*, *327*(11), 782–789. https://doi.org/10.1056/NEJM199209103271108

[4] Hassan Abed, Z. (2025). A comprehensive review of acyclovir: synthesis, antiviral mechanism, modifications, and innovative analytical techniques in pharmaceutical applications. *Chemical Review and Letters*, *8*(5), 967–980. https://doi.org/10.22034/crl.2025.492025.1487

[5] Saad, M. O., Habib, S. O., Alhomosy, A. M., Salem, I. M., & Hishari, O. M. (2025). Safety of different weight-based dosing strategies of intravenous acyclovir in obese patients: A retrospective cohort study. *Annals of Pharmacotherapy*, *59*(9), 801–808. https://doi.org/10.1177/10600280251318017

[6] Abdalla, S., Briand, C., Oualha, M., Bendavid, M., Béranger, A., Benaboud, S., ... & Hirt, D. (2020). Population pharmacokinetics of intravenous and oral acyclovir and oral valacyclovir in pediatric population to optimize dosing regimens. *Antimicrobial Agents and Chemotherapy*, *64*(12), 10–1128. https://doi.org/10.1128/aac.01426-20

[7] Patil, A. H., Singh, M. C., Musa, M. S., Mueid, M. A. Al, Reza, H. M., Enan, M. E., … & Patil, R. B. (2025). Identifying the worthy and compatible excipients through in-silico approach for rational formulation development: Insights into the acyclovir and trimethoprim formulations. *Journal of Pharmaceutical Innovation*, *20*(6), 283. https://doi.org/10.1007/s12247-025-10206-1

[8] Hakkimane, S. S., Paladugulu, D. V., Gaonkar, S. L., Karunakaran, K., Mudgal, P. P., Nayak, Y. N., & Guru, B. R. (2025). Biodegradable polymeric nano formulation of acyclovir and acyclovir prodrug for enhanced therapeutic efficacy against herpes simplex virus infections. *Research Square*, 1–26. https://doi.org/10.21203/rs.3.rs-7427697/v1

[9] Zarenezhad, E., Saleh, R. O., Osanloo, M., Iraji, A., Dehghan, A., Marzi, M., & Ghasemian, A. (2024). Nanoniosomes: Preparation, characterization, and insights into the skin cancer therapy (a review). *Russian Journal of Bioorganic Chemistry*, *50*(3), 855–869. https://doi.org/10.1134/S1068162024030348

[10] Salehan, F., Mohammadi, Y., Shieh, M., Askarizadeh, M., Rahmani, S., Alishahi, F., & Hheidari, A. (2025). Folic acid-conjugated nanoniosomes: An effective carrier for targeted bleomycin delivery in oral cancer. *Asian Pacific Journal of Cancer Biology*, *10*(1), 63–70. https://doi.org/10.31557/apjcb.2025.10.1.63-70

[11] Janakiraman, K., Sethuraman, V., & Jayaraj, G. (2025). A review on relevance of nanosomes in contemporary therapeutics: innovative approach to drug delivery. *BioNanoScience*, *15*(1), 191. https://doi.org/10.1007/s12668-024-01778-2

[12] Qian, H., Lv, J., & Hu, X. (2025). Development and evaluation of curcumin nano-niosomes for glioma-targeted therapy. *Scientific Reports*, *15*(1), 10520. https://doi.org/10.1038/s41598-025-95348-5

[13] Baniasad, A., Baei, M. S., & Tala-Tapeh, S. M. (2025). Chitosan-PEGylated niosomes and liposomes as biomacromolecule carriers for Alzheimer's disease treatment: Galantamine drug delivery carrier. *Materials Chemistry and Physics*, *352*, 132003. https://doi.org/10.1016/j.matchemphys.2025.132003

[14] Golestannejad, Z., Khozeimeh, F., Mehrasa, M., Mirzaeei, S., & Sarfaraz, D. (2022). A novel drug delivery system using acyclovir nanofiber patch for topical treatment of recurrent herpes labialis: A randomized clinical trial. *Clinical and Experimental Dental Research*, *8*(1), 184–190. https://doi.org/10.1002/cre2.512

[15] Pourhajibagher, M., Moghaddam, E. K., Moeininejad, M., Esboei, B. R., & Bahador, A. (2025). Ex vivo assessment of blue laser-activated berberine-loaded nanoniosomes in enhancing photodynamic therapy against Streptococcus mutans biofilm on tooth enamel. *Photodiagnosis and Photodynamic Therapy*, *56*, 105229. https://doi.org/10.1016/j.pdpdt.2025.105229

[16] Singh, J. P., Saini, G., Singh, B., & Tiwari, G. (2025). Nano-formulation approaches to enhance transdermal drug delivery-an updated review of nanovesicular carrier "transethosomes." *Pharmaceutical Nanotechnology*, *13*(4), 739–757. https://doi.org/10.2174/0122117385306281240427073651

[17] Smith, C. J., Eavis, H., Briggs, C., Henrici, R., Karpiyevich, M., Ansbro, M. R., ... & Artavanis-Tsakonas, K. (2025). Drug resistance–associated mutations in Plasmodium UBP-1 disrupt its essential deubiquitinating activity. *Journal of Biological Chemistry*, *301*(3), 1-11. https://doi.org/10.1016/j.jbc.2025.108266

[18] Shahbazi, R., Mirjafary, Z., Zarghami, N., & Saeidian, H. (2024). Efficient PEGylated magnetic nanoniosomes for co-delivery of artemisinin and metformin: A new frontier in chemotherapeutic efficacy and cancer therapy. *Scientific Reports*, *14*(1), 27380. https://doi.org/10.1038/s41598-024-78817-1

[19] Priprem, A., Johns, J. R., Limsitthichaikoon, S., Limphirat, W., Mahakunakorn, P., & Johns, N. P. (2017). Intranasal melatonin nanoniosomes: Pharmacokinetic, pharmacodynamics and toxicity studies. *Therapeutic Delivery*, *8*(6), 373–390. https://doi.org/10.4155/tde-2017-0005

[20] Motallebi, S. (2025). Advances in mesoporous silica nanoparticles for targeted and controlled drug delivery. *Biocompounds*, *2*(4), 212–225. https://doi.org/10.48313/bic.v2i4.49