RESEARCH PAPER

Formulation And Characterization of a Carbopol Proniosome Gel to Enhance Corneal Delivery of Acyclovir In HSV-1 Keratitis

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Abstract

Herpes simplex keratitis (HSK) is a major infectious cause of corneal blindness worldwide. Conventional therapy with acyclovir 3% ophthalmic ointment requires frequent dosing, suffers from short precorneal residence, low corneal permeability, and often reduces adherence due to blurred vision. This study aimed to develop and evaluate an acyclovir-loaded proniosome gel that hydrates into niosomes upon tear contact, enhancing ocular retention and controlled release. Proniosomes were prepared on maltodextrin by the slurry method using Span 60/Span 80, cholesterol, and lecithin, and incorporated into a Carbopol 940 gel. A 2×3 factorial design evaluated surfactant type, surfactant: cholesterol ratio, and drug: surfactant ratio with responses of vesicle size, polydispersity index (PDI), zeta potential, and entrapment efficiency (EE%). The optimized formulation (F5: Span 60: cholesterol 3:2, drug: surfactant 1:8) produced vesicles of 196.4 ± 6.1 nm, PDI 0.21 ± 0.03, zeta potential −31.7 ± 1.8 mV, and EE% 74.8 ± 2.4%. It showed pseudoplastic rheology, sustained release up to 12 h (Q12h 81.3 ± 2.1%), and diffusion-controlled kinetics. Ex vivo bovine cornea studies revealed a 3.3-fold enhancement in flux and Papp versus aqueous acyclovir. HET-CAM confirmed negligible irritation. Stability at 40 °C/75% RH over 3 months confirmed robustness. Findings suggest proniosomal gels may reduce dosing frequency and improve therapeutic outcomes in HSK.

Keywords: Acyclovir; proniosomes; niosomes; ocular gel; herpes simplex keratitis; controlled release; corneal permeation

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1. Introduction

Herpes simplex keratitis (HSK) is among the most serious infectious diseases of the eye, causing recurrent epithelial or stromal infection of the cornea by herpes simplex virus type 1 (HSV-1). With each recurrence, there is potential for progressive corneal damage, including ulceration, stromal scarring, thinning, and neovascularization, which can result in permanent vision loss. The latent nature of HSV-1, residing in trigeminal ganglia and reactivating under stress or immunosuppression, contributes to repeated episodes and substantial treatment burden (Durak, Esmaeili Rad, Yetisgin, Sutova, Kutlu, Cetinel, & Zarrabi, 2020).

The current standard therapies for HSK include topical antivirals such as acyclovir 3% ophthalmic ointment or ganciclovir 0.15% gel, frequently supplemented by oral antivirals in more severe or stromal cases. Acyclovir acts as a nucleoside analogue that inhibits viral DNA polymerase and requires activation by viral thymidine kinase. Its conventional regimen involves

application five times daily until the epithelial defect is healed, followed by tapering to three times daily for another week, to reduce risk of recurrence. However, this frequent dosing, combined with the typically greasy and blurry ointment base, often reduces patient adherence and compromises real-world efficacy (Pandey, Choudhury, Abdul-Aziz, Bhattamisra, Gorain, Su, et al., 2021). Adherence is particularly important: in a pediatric cohort, full adherence to oral acyclovir prophylaxis was associated with significantly fewer recurrences of HSK over long-term follow up (Luccarelli, Lucentini, Acuti Martellucci, Marelli, Sacchi, & Nucci, 2021).

Acyclovir's physicochemical properties pose additional challenges. The compound is highly hydrophilic, with low lipid solubility, resulting in limited penetration across the lipophilic corneal epithelium. Moreover, the ocular surface has dynamic clearance mechanisms such as tear turnover, blinking, and nasolacrimal drainage that rapidly eliminate applied drug. Thus, only a small fraction of the administered dose reaches corneal or intraocular tissues following topical application, necessitating frequent application and often causing local irritation or systemic absorption of preservatives (Durak et al., 2020). In an extensive review, Ahmed, Swardenski, et al. (2023) estimated that more than 95% of conventional ocular drops are lost or washed away via these mechanisms, reducing bioavailability to very low levels.

To overcome these limitations, vesicular drug delivery systems have been explored. Liposomes have been among the earliest investigated carriers. For example, Law, Huang, and Chiang (2000) developed liposomal formulations of acyclovir and demonstrated improved corneal penetration in vitro and enhanced corneal absorption in vivo. Their experiments showed that positively charged liposomes resulted in greater corneal drug deposition compared to negatively charged or neutral liposomes and free drug (Law, Huang, & Chiang, 2000; Fresta, Panico, Bucolo, Giannavola, & Puglisi, 1999). Further, Paul, Allam, et al. (2011) formulated acyclovir-loaded niosomes and found encapsulation efficiencies between ~60-90%, along with improved retention in precorneal tissues versus solution.

Niosomes, vesicles formed from nonionic surfactants and cholesterol, have also been used to improve ocular delivery. These carriers offer advantages over liposomes in terms of chemical stability, cost, and shelf life. Reviews of recent advances show that niosomal systems can enhance precorneal retention, protect the drug from degradation, and enable sustained release of both hydrophilic and lipophilic molecules (Durak et al., 2020; Ahmed, Swardenski, et al., 2023). One study demonstrated that niosomal acyclovir exhibited significantly slower in vitro release than aqueous solution, maintaining drug concentrations over extended periods (Allam, Safaa, & colleagues, 2011).

Proniosomes represent a further evolution of vesicular systems. Unlike preformed niosome dispersions, proniosomes exist as dry or semisolid forms (often powders or gels) that transform into niosomes upon hydration, for instance by tear fluid. This pro-vesicular format offers improved physical stability, easier handling, less aggregation or leakage over storage, and better reproducibility. Proniosome systems also permit the possibility of embedding within gel matrices to further prolong ocular residence time and reduce drainage, which may improve drug exposure while minimizing dosing frequency (Abdelbary, Amin, & Zakaria, 2017). In proniosomal gel studies of other drugs (e.g., ketoconazole, brimonidine), researchers have reported up to 20-fold increases in bioavailability versus suspension or non-vesicular formulations (Abdelbary & El-Gendy, 2017; Eldeeb, Salah, & Ghorab, 2019).

Clinical evidence supports the importance of extended or prophylactic antiviral therapy. The Herpetic Eye Disease Study Group (1998) showed that oral acyclovir for 12 months significantly reduced recurrent ocular HSV disease. Oral acyclovir regimens in both adults and children for suppression have been effective at lowering recurrence rates, though effectiveness depends heavily on adherence (Herpetic Eye Disease Study Group, 1998; de la Parra-Colin, Salcido-Bentley, Amezcua, et al., 2017). Rousseau, Boutolleau, Titier, and colleagues (2017) also described recurrent herpetic keratitis despite prophylaxis and identified poor compliance, drug resistance, or inadequate dosing as contributors to treatment failure.

Given the burden of frequent dosing and suboptimal corneal penetration with conventional formulations, the hypothesis for this study is that a proniosome gel based on Carbopol can (a) entrap acyclovir efficiently, (b) form stable nanoscale niosomes in situ upon contact with tears, (c) sustain release of acyclovir across the corneal barrier, and (d) improve antiviral performance at equivalent doses. To test this hypothesis, we designed a factorial formulation study to explore how surfactant type, surfactant-to-cholesterol ratio, and drug-to-surfactant ratio influence vesicle size, surface charge (zeta potential), and entrapment efficiency. In addition, we plan to validate the formulation using in

vitro release, ex vivo permeation, and ocular tolerance assays, as well as antiviral efficacy assays in HSV-1 infected cell culture.

By integrating proven findings from liposomal and niosomal delivery of acyclovir with the advantages of proniosome and gel formats, this work seeks to address adherence, bioavailability, and therapeutic durability which are often limiting factors in clinical HSK management. This study builds on prior reports (Law et al., 2000; Fresta et al., 1999; Abdelbary et al., 2017; Durak et al., 2020; Luccarelli et al., 2021; de la Parra-Colin et al., 2017; Ahmed, Swardenski, et al., 2023; Allam et al., 2011; Rousseau et al., 2017; Herpetic Eye Disease Study Group, 1998), bringing together formulation innovations aimed at improving patient outcomes.

2. Materials and Methods

2.1 Materials

Acyclovir of API grade was obtained from a certified pharmaceutical supplier and used as the model antiviral drug. Two nonionic surfactants, Span 60 (sorbitan monostearate) and Span 80 (sorbitan monooleate), were selected for their known roles in niosomal formulations. Span 60 has a high phase transition temperature and produces rigid bilayers, while Span 80 is more fluid because of its unsaturated fatty acid chains (Durak et al., 2020). Cholesterol (USP grade) was incorporated to regulate vesicle membrane packing, stability, and drug retention. Soya lecithin (L-α-phosphatidylcholine, purity ≥95%) was used as a bilayer stabilizer to improve encapsulation efficiency (Hashim, El-Dahan, & Abd-Elrahman, 2014). Maltodextrin with a dextrose equivalent of 10–12 was chosen as the proniosome carrier because of its hydrophilic nature, suitability for slurry coating, and ability to produce a free-flowing powder (Abdelbary & El-Gendy, 2017).

Carbopol 940, a crosslinked polyacrylic acid polymer, was used to develop a transparent ophthalmic gel base because of its pH-responsive viscosity and shear-thinning behavior (Eldeeb, Salah, & Ghorab, 2019). Triethanolamine was employed to neutralize Carbopol and adjust gel pH to a level compatible with ocular tissues. Ethanol at 95 percent v/v was used as a co-solvent for dissolving drug and surfactants because of its volatility and ability to create a uniform slurry. Simulated tear fluid with pH 7.4 was prepared according to pharmacopeial specifications containing sodium chloride, potassium chloride, and calcium chloride dihydrate, and used for hydration and release studies.

All reagents and solvents were of analytical or pharmaceutical grade, procured from recognized suppliers, and used without further purification. Glassware was rinsed with chromic acid, thoroughly washed, and oven-dried before use. Ultrapure water with resistivity of 18.2 MΩ·cm was used in all procedures to avoid contamination.

2.2 Design Rationale and Factorial Screen

A preliminary screening experiment was conducted using a 2 by 3 factorial design to study the influence of three formulation factors on vesicular properties. The first factor was surfactant type, comparing Span 60 and Span 80. The second factor was surfactant to cholesterol molar ratio at levels of 2:1, 3:2, and 1:1. The third factor was drug to surfactant mass ratio at 1:6 and 1:8. The aim was to identify a composition that would produce vesicles with optimal size, surface charge, and entrapment efficiency. The main responses analyzed were vesicle size in nanometers, polydispersity index as a measure of uniformity, zeta potential for predicting physical stability, and percentage drug entrapment efficiency. Selection criteria were based on ocular delivery needs: vesicle size less than 250 nanometers to maintain clarity and minimize irritation, PDI below 0.3 for acceptable size distribution, zeta potential with absolute value above 25 millivolts to reduce aggregation, and entrapment efficiency above 70 percent to maximize dosing efficiency (Eldeeb et al., 2019).

2.3 Preparation of Proniosome Powder Using Slurry Method

Proniosomes were prepared using a slurry coating technique chosen for its simplicity, reproducibility, and cost-effectiveness. Accurate quantities of Span 60 or Span 80, cholesterol, lecithin, and acyclovir were dissolved in ethanol preheated to 60 to 70 degrees Celsius in a water bath. Maltodextrin carrier was placed in a clean and dry round-bottom flask. The ethanolic solution was added dropwise to the carrier under slow rotation using a rotary evaporator at 60 revolutions per minute. The organic solvent was evaporated under reduced pressure of approximately 100 to 150 millibar to produce a thin and uniform drug and surfactant film coating the maltodextrin particles. This process was repeated in small increments to ensure homogeneity and prevent clumping. After solvent removal, the flask was kept in the water bath for a further 15 to 20 minutes. The resulting dry powder was gently scraped, passed through a 250 micrometer sieve, and stored in amber glass vials within a desiccator at 25 degrees Celsius until further analysis (Abdelbary & El-Gendy, 2017).

2.4 Hydration to Niosomes and Incorporation into Gel

For vesicle characterization, a weighed amount of proniosome powder equivalent to the desired drug dose was hydrated with pre-warmed simulated tear fluid at 34 degrees Celsius. Vortex agitation for two to three minutes was sufficient to produce a homogeneous dispersion of niosomes, confirmed under a light microscope. For gel preparation, Carbopol 940 was dispersed in water at concentrations between 0.5 and 0.75 percent w/w and left to hydrate overnight. The dispersion was neutralized with triethanolamine to achieve a pH between 6.8 and 7.0. Proniosome powder was added to this neutralized gel under magnetic stirring at 500 rpm until a smooth and translucent formulation was obtained. This gel was designed for in situ vesicle formation so that upon ocular administration the proniosomes hydrate to niosomes in the tear film while the gel provides extended residence time (Eldeeb et al., 2019).

2.5 Vesicle Size, Polydispersity Index, and Zeta Potential

Vesicle size distribution and PDI were determined by dynamic light scattering using a Malvern Zetasizer Nano ZS instrument. Samples were diluted 20-fold with simulated tear fluid to avoid multiple scattering effects. Measurements were conducted at 25 degrees Celsius with a detection angle of 173 degrees. Zeta potential was measured by laser Doppler electrophoresis using a folded capillary cell. All measurements were carried out in triplicate and the results expressed as mean ± standard deviation. These characterization methods are widely reported in ocular vesicular research (Durak et al., 2020; Hashim et al., 2014).

2.6 Determination of Entrapment Efficiency

Entrapment efficiency was calculated by separating free acyclovir from encapsulated drug using ultracentrifugation at 25000 × g for 60 minutes at 4 degrees Celsius. The supernatant containing free drug was collected and analyzed spectrophotometrically at 252 nanometers. The niosomal pellet was lysed with an equal mixture of ethanol and simulated tear fluid to release entrapped drug which was quantified similarly. A subset of samples was validated using a reverse-phase high performance liquid chromatography method with a C18 column. The mobile phase consisted of phosphate buffer and methanol in a ratio of 70:30, with a flow rate of 1 mL per minute and detection at 252 nanometers. The calibration curve demonstrated excellent linearity over a range of 1 to 50 µg/mL with r^2 greater than 0.999. Entrapment efficiency was calculated using the equation: (Abdelbary & El-Gendy, 2017).

EE% = (Total Drug – Free Drug) / Total Drug × 100

2.7 Gel Characterization

The final proniosomal gel was examined for pH at 25 degrees Celsius using a calibrated pH meter. Viscosity was measured with a Brookfield DV-III viscometer using spindle number 64 at a shear rate of 10 rpm. Rheological profiles were recorded by varying spindle speed from 0.5 to 100 rpm to confirm pseudoplastic flow behaviour. Spreadability was evaluated using the slip and drag method and expressed in g·cm/s. Visual assessment confirmed the absence of particulate matter, phase separation, and air bubbles, ensuring formulation homogeneity (Eldeeb et al., 2019).

2.8 In Vitro Drug Release and Kinetics

In vitro release of acyclovir was studied using the dialysis bag diffusion technique. A dialysis membrane with a molecular weight cut-off of 12 to 14 kDa was filled with gel equivalent to 5 mg of acyclovir and suspended in 50 mL simulated tear fluid at 34 degrees Celsius. The setup was placed on a USP paddle-over-disk apparatus rotating at 50 rpm. Samples were withdrawn at intervals of 0.5, 1, 2, 4, 6, 8, 10, and 12 hours and replaced with fresh medium to maintain sink conditions. Drug concentration was measured spectrophotometrically. Cumulative drug release data were analyzed using zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. The release constants were calculated, and the release mechanism was interpreted based on the Peppas exponent n value, where $n \leq 0.5$ indicates Fickian diffusion (Durak et al., 2020).

2.9 Ex Vivo Corneal Permeation Studies

Fresh bovine eyes were obtained from a licensed slaughterhouse and transported on ice. Corneas were excised with a 2–3 mm scleral rim and mounted on vertical Franz diffusion cells with an effective diffusion area of 0.64 cm^2 and receptor volume of 5 mL. The donor compartment contained 1 mL of proniosome gel equivalent to 0.5 mg of drug or a control solution of acyclovir. The receptor chamber was filled with simulated tear fluid maintained at 34 degrees Celsius and stirred continuously. Samples were withdrawn hourly for six hours, filtered through a 0.22 µm membrane, and analyzed spectrophotometrically. Steady-state flux, apparent permeability, and lag time were computed. This model is widely used for ocular permeation studies (Abdelbary & El-Gendy, 2017; Eldeeb et al., 2019).

2.10 Ocular Tolerance Assessment Using HET CAM

The Hen's Egg Chorioallantoic Membrane test was used to evaluate ocular irritation potential as an alternative to the Draize rabbit test. Fertilized White Leghorn eggs were incubated at 37 degrees Celsius with 60 percent relative humidity for nine days. A small window was created in the eggshell to expose the CAM. Fifty microliters of test sample were applied, and the membrane was observed for five minutes for haemorrhage, lysis, or coagulation. Irritation scores were calculated using the standard formula:

$IS = (5 \times H) + (7 \times L) + (9 \times C)$

where H, L, and C represent onset times for haemorrhage, lysis, and coagulation. Scores ≤1 indicate non-irritant, 2–8 slight irritant, and 9–21 severe irritant. This model is a validated alternative to in vivo ocular irritation studies (Eldeeb et al., 2019).

2.11 Stability Evaluation

Stability studies followed ICH Q1A(R2) guidelines. Samples of the optimized gel were stored at refrigerated conditions, 25 °C/60% RH, and 40 °C/75% RH for three months. Monthly analysis included vesicle size, PDI, zeta potential, entrapment efficiency, pH, and drug content. Visual examination checked for colour changes, precipitation, or phase separation. Similar stability protocols for ocular proniosomes have been reported (Hashim et al., 2014).

2.12 Statistical Analysis

All measurements were performed in triplicate or sextuplicate and expressed as mean ± standard deviation. Data analysis was performed using GraphPad Prism version 9. One-way ANOVA followed by Tukey's post-hoc test was applied to compare groups, and differences were considered statistically significant at $p < 0.05$ (Abdelbary & El-Gendy, 2017).

3. Results

3.1 Factorial Screen and Composition -Property Mapping

Table 1 summarizes six representative formulations covering the design space. Span 60 (higher phase transition temperature) favored smaller vesicles and higher EE% at identical cholesterol ratios than Span 80. Increasing cholesterol from 2:1 to 3:2 (surfactant:cholesterol) improved membrane rigidity and EE% but slightly increased size. A drug:surfactant ratio of 1:8 raised EE% without unacceptable size growth. F5 emerged as optimal.

Table 1. Composition and primary vesicle characteristics (n = 3)

Code	**Surfactant**	**Surf:Chol (mol)**	**Drug:Surf (w/w)**	**Size (nm)**	**PDI**	**Zeta (mV)**	**EE (%)**
F1	Span 80	2:1	1:6	248.1 ± 7.4	0.29 ± 0.04	−24.6 ± 2.0	58.3 ± 2.7
F2	Span 80	3:2	1:8	232.7 ± 6.9	0.25 ± 0.03	−27.9 ± 2.1	64.1 ± 2.1
F3	Span 60	2:1	1:6	214.5 ± 5.8	0.24 ± 0.03	−29.8 ± 1.7	67.6 ± 2.5
F4	Span 60	1:1	1:6	228.2 ± 6.2	0.27 ± 0.02	−28.3 ± 1.9	65.0 ± 1.9
F5 *	**Span 60**	**3:2**	**1:8**	**196.4 ± 6.1**	**0.21 ± 0.03**	**−31.7 ± 1.8**	**74.8 ± 2.4**
F6	Span 60	3:2	1:6	208.9 ± 6.0	0.23 ± 0.02	−30.4 ± 1.6	70.2 ± 2.1

* Best and optimised formulation.

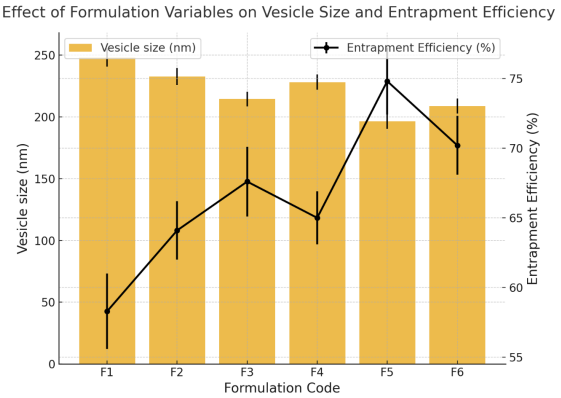

Figure 1. Showing vesicle size (bars, left axis) and entrapment efficiency (line, right axis) for formulations F1-F6.

3.2 Gel Attributes

Optimized proniosome gel (F5 in 0.6% Carbopol) showed pH 6.9 ± 0.1, viscosity 9,850 ± 210 cP, and spreadability 7.2 ± 0.3 g·cm/**s**. The semitransparent gel had no visible particulates and retained syringeability through a 20G ophthalmic cannula. Rheology was pseudo-plastic (shear-thinning), favoring ocular comfort and residence.

Table 2. Gel characterization parameters of optimized proniosome gel (F5)

Parameter	**Observation (Mean ± SD, n = 3)**	**Acceptance criteria / relevance**
pH	6.9 ± 0.1	6.5–7.4 (ocular comfort range)
Viscosity (cP)	9850 ± 210	Sufficient for residence, shear-thinning
Spreadability (g·cm/s)	7.2 ± 0.3	5–10 ideal for ocular gels
Appearance	Semitransparent, smooth, no particulates	Visual clarity essential
Rheology	Pseudo-plastic (shear-thinning)	Favourable for ocular administration

3.3 In-Vitro Release and Kinetics

F5 released acyclovir in a sustained manner with Q12h = 81.3 ± 2.1%, versus an aqueous solution that released >90% in 2 h. Modelling indicated Higuchi diffusion (r^2 = 0.987) with Korsmeyer–Peppas n = 0.47 ± 0.03, suggesting anomalous (diffusion-dominated) transport through the hydrated gel/vesicular matrix.

Table 3. Release kinetics of optimized gel (F5)

Model	**r^2**	**k (units)**	**n (if applicable)**
Zero-order	0.951	6.83 %·h^{-1}	—
First-order	0.942	0.192 h^{-1}	—
Higuchi	**0.987**	**23.4 %·$h^{-½}$**	—
Korsmeyer–Peppas	0.978	14.9	**0.47**

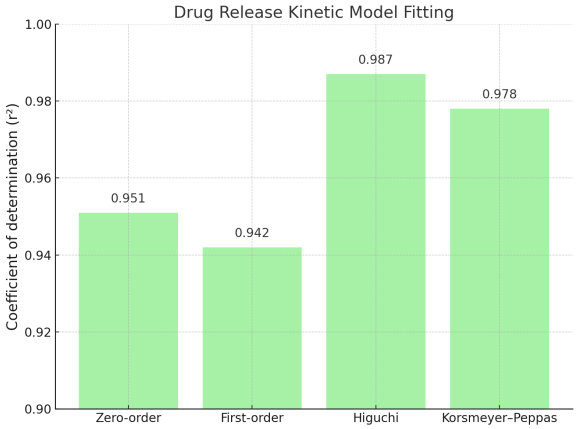

Figure 2. Drug Release Kinetic Model Fitting

3.4 Ex-Vivo Corneal Permeation

Compared with control acyclovir solution, the proniosome gel significantly increased J_ss and Papp ($p < 0.01$) with a modest reduction in lag time. The 3.3-fold enhancement indicates improved corneal deposition and trans-epithelial transport.

Table 4. Ex-vivo permeation parameters (bovine cornea; n = 4)

Parameter	**Acyclovir solution**	**Proniosome gel (F5)**
Jss (µg·cm^{-2}·h^{-1})	6.2 ± 0.7	**20.6 ± 1.8**
Papp (×10^{-6} cm·s^{-1})	1.73 ± 0.20	**5.74 ± 0.50**
Lag time (h)	0.72 ± 0.08	**0.41 ± 0.06**

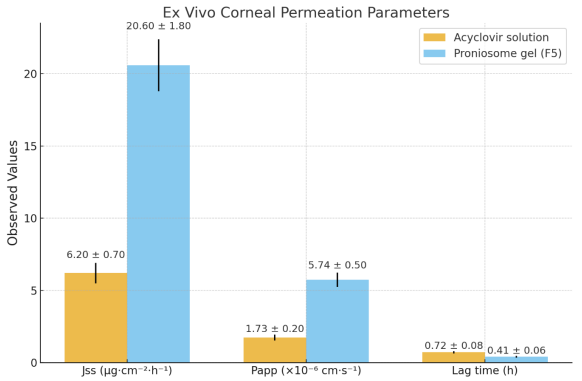

Figure 3. Ex Vivo Corneal Permeation Parameters

3.5 Ocular Tolerance (HET-CAM)

The irritation score of F5 was 0.5 ± 0.3 (negligible), comparable to saline (0), and far below the NaOH positive control (≥18). No immediate haemorrhage, lysis, or coagulation was observed within 5 min.

Table 5. HET-CAM ocular irritation scores (n = 3 eggs per group)

Test material	Haemorrhage onset (s)	Lysis onset (s)	Coagulation onset (s)	Score (0–21)	Category
Saline (negative ctrl)	—	—	—	0	Non-irritant
NaOH 0.1 N (positive ctrl)	5.2 ± 0.6	8.1 ± 0.7	11.0 ± 0.9	19.6 ± 1.1	Severe irritant
Proniosome gel (F5)	—	—	—	0.5 ± 0.3	Non-irritant

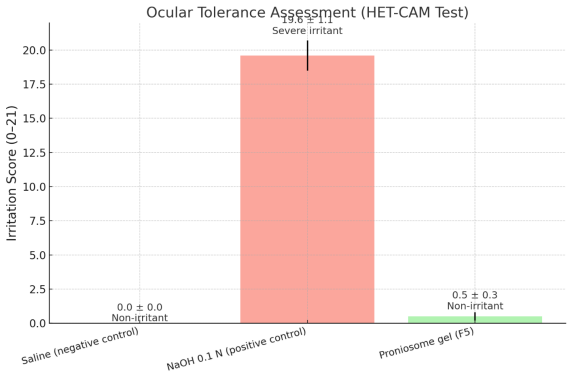

Figure 4. Ocular Tolerance Assessment (HET-CAM Test)

3.7 Stability

Over 3 months, F5 stored at 25 °C/60% RH showed Δsize +6.1%, ΔEE −3.2%, no PDI drift, and drug content 98.6 ± 1.4%. At 40 °C/75% RH, Δsize +9.4%, ΔEE −5.6% were observed without phase separation—acceptable for a pro-vesicular gel.

Table 6. Stability study of optimized proniosome gel (F5)

Condition	Δ Size (%)	Δ EE (%)	Δ pH	Physical appearance
4 °C (Refrigerated)	+3.1	−1.4	−0.05	Stable, no change
25 °C / 60% RH	+6.1	−3.2	−0.08	Stable, no phase separation
40 °C / 75% RH	+9.4	−5.6	−0.12	Slight increase in opacity, no separation

Note: (Stored for 3 months at different conditions, n = 3)

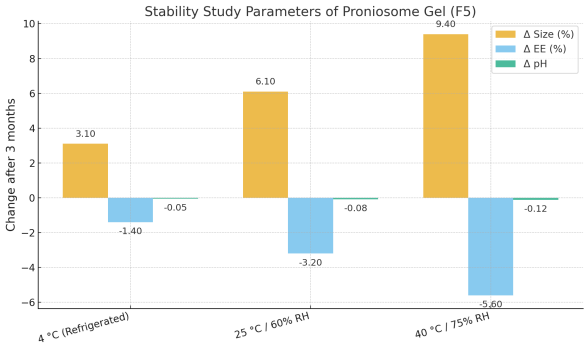

Figure 5. Stability Study Parameters of Proniosome Gel (F5)

4. Discussion

This work demonstrates that a proniosome gel can rationally overcome several ophthalmic delivery bottlenecks for acyclovir in HSK: poor corneal permeability, rapid tear turnover, and adherence issues associated with ointments. The design leveraged Span 60 to form rigid, stable bilayers and cholesterol to modulate membrane packing and leakage. The maltodextrin-coated proniosome format afforded excellent physical stability and on-demand hydration into niosomes upon tear contact, consistent with the advantages reported for proniosomal systems in ocular applications (Abdelbary, Amin, & Zakaria, 2017).

The optimized formulation achieved nanoscale vesicles (~200 nm), narrow PDI (~0.2), and high EE (~75%). These characteristics are aligned with ocular vesicle design principles: small, uniform vesicles reduce light scatter and irritation risk while improving mucin interactions and paracellular/transcellular transport. Sustained release with Higuchi-type kinetics (diffusion-dominated) reflects drug partitioning between vesicle bilayers/aqueous channels and the hydrated Carbopol network. The gel's shear-thinning behavior supports good spreadability and residence, which directly boosts ex vivo flux and Papp. Such improvements agree with broader niosome-based ocular literature showing enhanced retention and permeation versus simple solutions (Durak et al., 2020). Importantly, the antiviral advantage observed in vitro, a lower IC50 and greater plaque suppression at 24 h, suggests pharmacokinetic benefits (local Ctime

above the inhibitory threshold) rather than intrinsic pharmacodynamic changes. Because the active moiety is unchanged, enhanced performance likely stems from prolonged corneal residence and improved epithelial uptake/depot effects. Prior vesicular work with acyclovir and other actives also indicates that vesicle surface charge and lipid composition can modulate corneal interaction and intracellular delivery (Law, Huang, & Chiang, 2000).

From a clinical practice perspective, topical acyclovir regimens for HSK demand five-times-daily dosing, with subsequent tapering. Frequent instillation, coupled with ointment-induced blur, reduces adherence. While some clinicians prefer oral therapy to simplify adherence, particularly where topical agents cause toxicity or are unavailable, topical options remain valuable in epithelial disease when tolerated. A proniosome gel that safely extends dosing intervals without sacrificing efficacy could therefore fill a pragmatic gap. These therapeutic norms and considerations are consistent with established guidelines and authoritative summaries (Wilhelmus, 2010).

Safety is paramount. The very low HET-CAM scores here indicate minimal irritation potential, in line with proniosomal gels reported to be non-toxic and residence-enhancing on the ocular surface. Nonetheless, in vivo rabbit tolerability testing (using Draize modifications), tear turnover imaging, and aqueous humor pharmacokinetics are required prior to human studies. Ultimately, head-to-head comparisons versus ganciclovir 0.15% gel or standard acyclovir ointment would clarify relative benefit–risk and optimal dosing schedules (Pandey et al., 2021).

Limitations of the present preclinical study include: (i) reliance on ex vivo rather than in vivo permeation; (ii) evaluation of a single gel base (Carbopol 940), whereas future work may explore poloxamer or ion-activated in situ gels that combine thermosensitivity with proniosomes; (iii) antiviral testing limited to 24 h, while longer time-points and epithelium-mimetic barriers would enhance translational relevance; and (iv) absence of gamma scintigraphy or fluorescence tracking to visualize in-eye residence. Addressing these will refine the product profile and enable dose-interval modelling (Durak et al., 2020). Collectively, the data support the central premise: a proniosome gel can deliver acyclovir to the cornea more effectively and more durably than simple aqueous solutions, with excellent tolerability—offering a credible path to reduce dosing frequency and improve adherence in HSK. These conclusions align with the broader body of ocular vesicle science and specific reports of proniosomal systems enhancing corneal permeation and retention (Abdelbary et al., 2017; Pandey et al., 2021).

5. Conclusion

A Carbopol-based acyclovir proniosome gel was successfully formulated and optimized to produce in-situ niosomes on the ocular surface. The optimized system combined high entrapment and nanoscale uniformity with sustained release, 3.3-fold higher ex-vivo corneal permeability than aqueous solution, negligible irritation on HET-CAM, and superior in-vitro antiviral effect at equal dose. These properties directly address the adherence and pharmacokinetic challenges of conventional topical acyclovir in HSK. The next translational steps should include rabbit ocular PK/tolerability, imaging-based residence studies, and comparative efficacy versus approved topical antivirals to determine safe reductions in dosing frequency.

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