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Development and Evaluation of Acetazolamide-Loaded Nanospanlastic Gel for Enhanced Ocular Delivery

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Abstract:

Background: Acetazolamide, a potent antiglaucoma agent, lacks effective topical ocular delivery due to low aqueous solubility, corneal permeability, and short elimination from the precorneal area, hence low bioavailability and frequent dosing.

Aim: The current study aims to establish an acetazolamide-loaded nanospanlastic ocular gel to improve corneal permeability, increase ocular residence time, and provide sustained release for the therapy of glaucoma.

Methodology: Nanospanlastic vesicles were formed using the ethanol injection method. The drug purity, compatibility, and preformulation studies were carried out. The optimized nanospanlastics were characterized, and the final product in the form of a mucoadhesive gel was prepared. The physicochemical studies were done, followed by in vitro drug release, ex vivo corneal permeation, and stability studies.

Results: The optimized formulation had a particle size of 154.8 ± 2.11 nm, zeta potential of −28.6 ± 0.92 mV, and entrapment efficiency of 79.65 ± 1.14%. Ocular gel showed physiological pH, adequate viscosity, reproducible drug content, controlled drug release for a period of up to 12 h, and improved corneal permeation. The stability studies confirmed the integrity of the formulation during three months.

Conclusion: The developed acetazolamide-loaded nanospanlastic gel is a stable and effective ocular delivery system that may provide improved management of glaucoma.

Keywords: Acetazolamide; Corneal permeation; Glaucoma therapy; Nanospanlastics; Ocular drug delivery; Sustained drug release

Introduction:

Glaucoma is a chronic and progressive optic neuropathy characterized by the irreversible degeneration of retinal ganglion cells and corresponding visual field loss [1]. It remains one of the most common causes of irreversible blindness in the world, with prevalence expected to increase significantly as the population ages globally. The most significant and modifiable risk factor associated with both the onset and progression of glaucoma remains elevated IOP. Its effective control therefore remains the primary goal of therapeutic intervention [2]. Despite the availability of multiple pharmacological, laser, and surgical interventions, long-term medical therapy remains the cornerstone of the glaucoma management.

Topical ophthalmic delivery is the most preferred mode of administering drugs in the treatment of glaucoma as it is non-invasive and easy to use [3]. However, conventional eyed-drops face important drawbacks in their application to treat glaucoma, including rapid clearance from the preocular area, low permeability through the cornea, short term of stay, and low bioavailability in the eye. In general, only a low amount of the administered dose is available to the intraocular tissues owing to physiological obstructions such as tear turnover, blinking, nasolacrimal drainage, and the multi-layered structure of the cornea [4, 5]. Such drawbacks result in increased frequencies of doses, hence poor compliance and adverse systemic side effects, especially among older patients requiring long-term treatment.

Acetazolamide (AZM), a highly potent inhibitor of carbonic anhydrase, is efficient in lowering IOP by inhibiting the formation of AH [6]. However, when acetazolamide is taken orally, it has unacceptable side effects, including metabolic acidosis, paresthesia, and gastrointestinal side effects [7]. The application of acetazolamide topically is considered to be an emerging area, and its low solubility, permeability, and classification as a BCS class IV compound make it very difficult to show its efficacy [8,9]. Therefore, there is an urgent need to develop a novel ocular delivery system that will improve its solubility, permeability, and retention at the ocular surface.

Nanospanlastics, or elastic nanovesicular carriers consisting of non-ionic surfactants and edge activators, are observed to be promising carrier systems for ophthalmic drug delivery [10]. Nanospanlastics possess improved elasticity, which facilitates better corneal barrier penetration, and the vesicular nature ensures efficient entrapment of poorly soluble drugs. Nanospanlastics also provide stability, sustained drug release, and improved ocular bioavailability. Coating the mucoadhesive gel formulation containing these nanovesicles further extends the precorneal residence time, minimizes drug loss due to tear drainage, and improves patient compliance.

Thus, the present study focuses on the development and evaluation of acetazolamide-loaded nanospanlastic gels to enhance ocular delivery during glaucoma therapy. The primary aim is to overcome the limitations associated with conventional dosage forms, such as eye drops, by improving corneal permeation, ocular bioavailability, sustained release, and reducing the overall systemic bioavailability to ensure safer and more effective therapy for the management of glaucoma.

Materials and Methods

Materials

Acetazolamide (AZM) was used as the model antiglaucoma drug. Span 60 (sorbitan monostearate) and Tween 80 (polysorbate 80) were employed as the vesicle-forming surfactant and edge activator, respectively [11]. Carbopol 934P and hydroxypropyl methylcellulose (HPMC K4M) were used as gelling and mucoadhesive agents [12]. All chemicals and solvents were of analytical grade and used without further purification. Distilled water was prepared in-house.

Preformulation Studies

Preformulation studies were conducted to evaluate the physicochemical properties of acetazolamide and its compatibility with selected excipients. These studies included organoleptic evaluation, solubility analysis in various solvents, melting point determination, loss on drying, and partition coefficient determination using the shake-flask method. Drug identity and compatibility were confirmed using ultraviolet (UV) spectroscopy, Fourier transform infrared (FTIR) spectroscopy.

Preparation of Acetazolamide-Loaded Nanospanlastic Vesicles

Nanospanlastic vesicles were prepared using the ethanol injection method. Acetazolamide and Span 60 were dissolved in absolute ethanol to form the organic phase, while Tween 80 was dissolved in preheated distilled water to form the aqueous phase. The organic phase was injected dropwise into the aqueous phase under continuous magnetic stirring, resulting in spontaneous formation of nanospanlastic vesicles. The dispersion was subsequently probe-sonicated to reduce vesicle size and improve homogeneity, followed by overnight storage at 4 °C for stabilization.

Optimization and Characterization of Nanospanlastics

Formulation variables were optimized using a Box–Behnken experimental design. The prepared nanospanlastics were characterized for particle size, polydispersity index (PDI), and zeta potential using dynamic light scattering. Vesicle morphology was examined by scanning electron microscopy (SEM). Entrapment efficiency was determined by separating unentrapped drug through ultracentrifugation and quantifying acetazolamide spectrophotometrically.

Preparation of Nanospanlastic Ocular Gel

The optimized nanospanlastic dispersion was incorporated into a mucoadhesive gel base prepared by hydrating Carbopol 934P and HPMC K4M in distilled water. The polymers were allowed to swell overnight to obtain a uniform gel. The nanospanlastic dispersion was gently incorporated into the gel base to prevent vesicle rupture. The pH was adjusted to the physiological ocular range (6.8–7.4), and isotonicity was maintained.

Evaluation of Nanospanlastic Gel

The prepared nanospanlastic gel was evaluated for appearance, pH, viscosity, spreadability, and drug content uniformity. In vitro drug release studies were performed using suitable diffusion models to assess release behavior. Ex vivo corneal permeation studies were conducted using excised animal cornea to evaluate transcorneal drug transport. Stability studies were carried out according to ICH guidelines to assess the physical and chemical stability of the formulation.

Solubility Studies

The solubility profile of acetazolamide was determined in various solvents, including distilled water, polyethylene glycol 400, ethanol, propylene glycol, and phosphate buffer solutions of different pH values. Excess quantities of the drug were added to each solvent and shaken for a specified duration at room temperature to achieve equilibrium. After filtration, the concentration of dissolved drug was analyzed spectrophotometrically. The solubility data obtained were used to guide the selection of suitable solvents and formulation strategies for enhancing drug incorporation and release.

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using appropriate software tools. Optimization of formulation variables was carried out using a Box–Behnken experimental design to evaluate the influence of independent variables on particle size, entrapment efficiency, and stability of nanospanlastic vesicles. Analysis of variance (ANOVA) was applied to assess the statistical significance of the model and formulation variables, with a p-value < 0.05 considered statistically significant. Release and permeation data were analyzed to determine reproducibility and consistency of the developed formulation.

Results

The result of the present study demonstrates the experimental outcomes obtained from the comprehensive evaluation of acetazolamide and the developed nanospanlastic ocular gel formulation which include physicochemical characterization of the drug, optimization and characterization of nanospanlastic vesicles, formulation assessment of the ocular gel, and evaluation of in vitro drug release, ex vivo corneal permeation, and stability parameters. Table 1 summarizes the organoleptic properties of acetazolamide and demonstrates full compliance with pharmacopoeial specifications. The drug was observed to be a fine crystalline, white, and odorless powder with a characteristic bitter taste, which is consistent with its official monograph standards. These observations confirm the identity and purity of acetazolamide and indicate the absence of any abnormal physical characteristics that could suggest contamination or degradation. The conformity of all evaluated organoleptic parameters with pharmacopoeial requirements supports the suitability of acetazolamide for further formulation and development studies.

Table 1. Organoleptic Properties of Acetazolamide

Property	Observation	Pharmacopoeial Specification	Compliance
Appearance	Fine crystalline powder	Crystalline powder	Complies
Color	White	White	Complies
Odor	Odorless	Odorless	Complies
Taste	Bitter	Bitter	Complies

Table 2 presents the solubility profile of acetazolamide in different media and highlights its limited aqueous solubility. The drug exhibited poor solubility in distilled water (0.69 mg/mL), which is indicative of its inherent solubility challenges and justifies the need for a solubility-enhancing delivery system. A comparatively higher solubility was observed in simulated tear fluid (1.43 mg/mL) and phosphate buffer (pH 7.4) (1.51 mg/mL), suggesting improved dissolution in physiological and buffered environments. These findings indicate that while acetazolamide shows slightly better solubility under ocular physiological conditions, its overall low solubility remains a significant limitation for conventional ocular formulations, thereby supporting the rationale for developing a nanospanlastic-based delivery system to enhance drug solubilization and bioavailability.

Table 2. Solubility Profile of Acetazolamide

Solvent / Medium	Absorbance ± SD (n=3)	Solubility (mg/mL)
Distilled water	0.221 ± 0.015	0.69
Simulated Tear Fluid (STF)	0.676 ± 0.017	1.43
Phosphate buffer (pH 7.4)	0.499 ± 0.036	1.51

Table 3 presents the melting point determination of acetazolamide carried out over three independent trials, revealing melting ranges between 257 and 264 °C with mean melting point values of 260.0 °C, 259.0 °C, and 261.5 °C, respectively. The narrow melting ranges observed across all trials indicate a high degree of crystallinity and uniformity of the drug sample. The close agreement among the mean melting point values further demonstrates the reproducibility of the results and confirms the thermal stability of acetazolamide under the experimental conditions. Importantly, the observed melting point range is consistent with reported pharmacopoeial specifications, suggesting that the drug is free from significant impurities or degradation products, as the presence of such contaminants typically results in melting point depression or broadening. These findings confirm the purity and identity of acetazolamide and establish its suitability for subsequent formulation development and thermal processing during the preparation of nanospanlastic vesicles and ocular gel systems.

Table 3. Melting Point of Acetazolamide

Trial	Melting Range (°C)	Mean Melting Point (°C)
1	258–263	260.0
2	257–261	259.0
3	259–264	261.5

Table 4 summarizes the loss on drying results of acetazolamide, showing a moisture content of 0.0244%, which is well below the pharmacopoeial limit of not more than 0.5%. The extremely low loss on drying value indicates minimal residual moisture and confirms the dry and stable nature of the drug substance. Low moisture content is essential for ensuring the chemical stability of acetazolamide, as excess moisture can promote degradation and adversely affect flow and compaction properties. The compliance of the observed value with pharmacopoeial specifications confirms the suitability of acetazolamide for formulation development and supports its stability during processing and storage.

Table 4. Loss on Drying of Acetazolamide

Parameter	Observation	Pharmacopoeial Limit	Compliance
Loss on Drying (%)	0.0244	NMT 0.5%	Complies

Table 5 presents the partition coefficient of acetazolamide determined using the n-octanol and phosphate buffer (pH 7.4) system, yielding a mean partition coefficient (P) value of 4.00 ± 0.21 and a corresponding log P value of 0.60 ± 0.02. The observed log P value indicates a moderate lipophilic character of acetazolamide, suggesting its ability to partition between aqueous and lipid phases. This physicochemical property is favorable for incorporation into vesicular drug delivery systems, as moderate lipophilicity supports efficient entrapment within the lipid bilayer while maintaining sufficient aqueous solubility. Additionally, the partition behavior implies potential for improved corneal permeation, as the corneal epithelium favors drugs with balanced hydrophilic–lipophilic characteristics. These results further justify the selection of a nanospanlastic-based delivery system to enhance ocular bioavailability of acetazolamide.

Table 5. Partition Coefficient of Acetazolamide

System	P (Mean ± SD)	Log P (Mean ± SD)
n-Octanol: Phosphate Buffer (pH 7.4)	4.00 ± 0.21	0.60 ± 0.02

Table 6 summarizes the physicochemical characteristics of the optimized acetazolamide-loaded nanospanlastic formulation. The mean particle size of 154.8 ± 2.11 nm indicates the successful formation of nanosized vesicles, which is desirable for ocular drug delivery as smaller particles enhance corneal penetration and provide improved surface contact with ocular tissues. The low standard deviation reflects uniformity and reproducibility of the formulation process. The zeta potential value of −28.6 ± 0.92 mV suggests sufficient electrostatic repulsion between vesicles, contributing to good colloidal stability and minimizing the risk of aggregation during storage. Additionally, the polydispersity index (PDI) value of 0.26 ± 0.02 indicates a narrow particle size distribution, confirming the homogeneity of the nanospanlastic system. Collectively, these results demonstrate that the optimized formulation possesses appropriate size, stability, and uniformity, making it suitable for incorporation into an ocular gel and further evaluation for enhanced ocular drug delivery.

Table 6. Physicochemical Characteristics of Optimized Nanospanlastics

Parameter	Value (Mean ± SD, n=3)
Particle size (nm)	154.8 ± 2.11
Zeta potential (mV)	−28.6 ± 0.92

Table 7 presents the entrapment efficiency and drug loading capacity of the optimized acetazolamide-loaded nanospanlastic formulation. The formulation exhibited a high entrapment efficiency of 79.65 ± 1.14%, indicating effective incorporation of acetazolamide within the nanospanlastic vesicles. This high entrapment can be attributed to the affinity of the drug for the vesicular bilayer and the optimized surfactant–edge activator composition. The drug loading value of 68.85 ± 0.15% reflects an adequate amount of drug encapsulated relative to the total formulation weight, which is essential for achieving therapeutic drug levels while minimizing excipient load. The low standard deviation values for both parameters indicate good reproducibility of the formulation process. Overall, these results demonstrate the suitability of nanospanlastic vesicles as an efficient carrier system for acetazolamide in ocular drug delivery applications.

Table 7. Entrapment Efficiency and Drug Loading

Parameter	Value (Mean ± SD, n=3)
Entrapment Efficiency (%)	79.65 ± 1.14
Drug Loading (%)	68.85 ± 0.15

Table 8 summarizes the scanning electron microscopy (SEM) observations of the optimized acetazolamide-loaded nanospanlastic formulation. The vesicles exhibited a well-defined spherical shape with smooth and intact surfaces, indicating successful vesicle formation and structural integrity. The absence of cracks or surface irregularities suggests stability of the vesicular membrane and uniform surfactant distribution. The observed vesicle size range of 120–180 nm is consistent with particle size analysis results, further confirming the nanoscale dimensions of the formulation. Minimal aggregation was observed, which can be attributed to adequate surface charge and stabilization of the vesicles. Overall, the SEM findings corroborate the physicochemical characterization data and confirm the suitability of the nanospanlastic vesicles for ocular drug delivery.

Table 8. SEM Observation of Optimized Nanospanlastics

Feature	Observation
Vesicle shape	Spherical, well-defined
Surface morphology	Smooth, intact, no cracks or pores
Vesicle size range	120–180 nm
Aggregation	Minimal

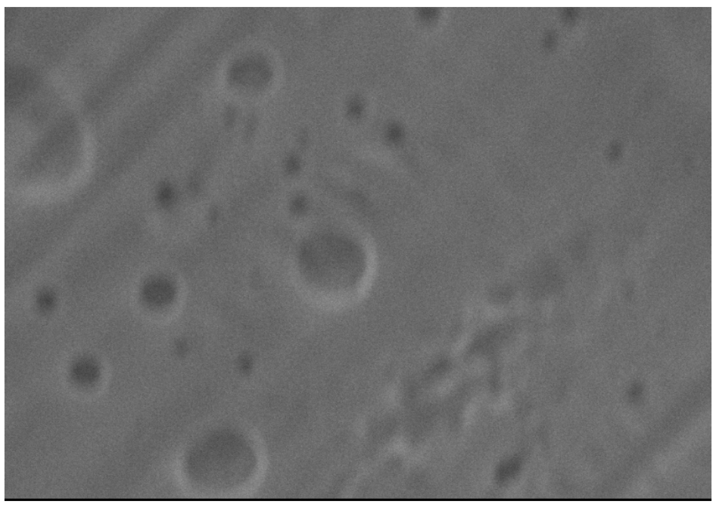

Figure 1. SEM Analysis of Optimized Nanospanlastics

The UV–visible spectroscopic analysis of acetazolamide (Table 9) demonstrated a characteristic sharp absorption peak at 266 ± 1 nm for the pure drug, confirming its identity. The optimized nanospanlastic formulation exhibited the same λmax without any significant shift, indicating the absence of drug–excipient interactions during vesicle formation. Similarly, the nanospanlastic gel retained the characteristic absorption peak at 266 ± 1 nm, confirming that acetazolamide remained chemically stable after incorporation into the gel matrix. Overall, the results presented in Table 9 indicate that the formulation process did not alter the chemical integrity of acetazolamide and that the drug was compatible with the selected excipients.

Table 9. UV–Visible Spectroscopic Analysis of Acetazolamide

Sample	λmax (nm)	Observation	Interpretation
Pure acetazolamide	266 ± 1	Sharp absorption peak	Confirms drug identity
Optimized nanospanlastics	266 ± 1	No shift in λmax	No drug–excipient interaction
Nanospanlastic gel	266 ± 1	Peak retained	Drug stability maintained

The FTIR spectra of pure acetazolamide and the optimized nanospanlastic formulation (Table 10) exhibited characteristic absorption bands corresponding to the functional groups of acetazolamide, including N–H stretching (~3290 cm^{-1}), C=O stretching (~1675 cm^{-1}), S=O stretching (~1145 cm^{-1}), C–N stretching (~1320 cm^{-1}), and aromatic C–H stretching (~3060 cm^{-1}). These characteristic peaks were retained in the nanospanlastic formulation with only minor shifts in wavenumber, which can be attributed to physical interactions or changes in the microenvironment rather than chemical modification. The absence of peak disappearance or formation of new peaks indicates that the chemical structure of acetazolamide remained intact and that no significant drug–excipient interactions occurred during formulation, confirming the compatibility and stability of acetazolamide within the nanospanlastic system.

Table 10. FTIR Spectral Characterization of Acetazolamide and Nanospanlastic Formulation

Functional group	Pure AZM (cm^{-1})	Formulation (cm^{-1})	Assignment
N–H stretching	~3290	~3285	Amide N–H
C=O stretching	~1675	~1670	Carbonyl group
S=O stretching	~1145	~1142	Sulfonamide group
C–N stretching	~1320	~1318	Amide linkage
Aromatic C–H	~3060	~3058	Aromatic ring

Table 11 presents the physicochemical characteristics of the nanospanlastic ocular gel, providing insight into its physical quality and suitability for ocular use. The gel is described as having a clear and homogeneous appearance, which indicates uniformity in its composition without phase separation or aggregation of particles. Its color is transparent, reinforcing the visual purity of the formulation, and the clarity is noted as clear, suggesting the absence of turbidity or cloudiness that could affect patient comfort or dosing accuracy. Additionally, the gel shows no grittiness, implying a smooth texture that would be gentle on the sensitive ocular surface. Overall, these observations collectively indicate that the gel has a visually appealing, stable, and patient-friendly formulation appropriate for ophthalmic application.

Table 11. Physicochemical Characteristics of Nanospanlastic Ocular Gel

Parameter	Observation
Appearance	Clear, homogeneous
Color	Transparent
Clarity	Clear
Grittiness	Absent

Table 12 presents the pH and drug content uniformity of the nanospanlastic ocular gel, parameters that are critical for assessing its physicochemical stability and suitability for ocular administration. The formulation exhibited a pH of 7.1 ± 0.2, which is closely aligned with the physiological pH of the human eye, indicating that the gel is likely to be non-irritant and well-tolerated upon topical application. Maintaining a pH near neutrality is essential for minimizing ocular discomfort and ensuring compatibility with the sensitive ocular tissues. Furthermore, the drug content was determined to be 98.6 ± 1.1%, reflecting a high degree of uniformity and demonstrating that the formulation can reliably deliver consistent doses of the active pharmaceutical ingredient. Collectively, these findings suggest that the nanospanlastic ocular gel possesses both chemical stability and formulation reliability, making it a suitable candidate for effective and safe ophthalmic drug delivery.

Table 12. pH and Drug Content Uniformity of Nanospanlastic Ocular Gel

Parameter	Value (Mean ± SD)
pH	7.1 ± 0.2
Drug content (%)	98.6 ± 1.1

Table 13 presents the viscosity and spreadability characteristics of the nanospanlastic ocular gel, which are critical parameters influencing its application and retention on the ocular surface. The gel exhibited a viscosity of 4,850 ± 120 cPs, indicating a sufficiently thick consistency that can enhance the residence time of the formulation in the eye, thereby potentially improving drug absorption and therapeutic efficacy. Despite its relatively high viscosity, the gel demonstrated a spreadability of 18.2 ± 0.9 g·cm/s, suggesting that it can be easily applied and uniformly distributed over the ocular surface without causing discomfort. Together, these findings indicate that the formulation strikes an appropriate balance between mechanical stability and ease of administration, ensuring both patient acceptability and effective ocular drug delivery.

Table 13. Viscosity and Spreadability of Nanospanlastic Ocular Gel

Parameter	Value (Mean ± SD)
Viscosity (cPs)	4,850 ± 120
Spreadability (g·cm/s)	18.2 ± 0.9

Table 13 displays in vitro drug release profiles of pure AZM solution, nanospanlastics, and nanospanlastic ophthalmic gel products over a 12-hour period. As can be observed in Table 13, pure AZM solution has demonstrated a faster drug release profile, where 42.3 % ± 1.8 % released at 1 hour increased to 88.4 % ± 2.6 % at 4 hours. In contrast, nanospanlastic has a relatively slower rate of in vitro drug release, where 28.6 % ± 1.4 % released at 1 hour increased to 85.2 % ± 2.7 % at 8 hours, indicating a sustained release profile. Therefore, it can be noted in Table 13, at 1 hour, 18.2 % ± 1.1 % released, and at 12 hours, 88.7 % ± 2.9 % released from a nanospanlastic ophthalmic gel. Therefore, it seems to infer a slower rate of release from a gel matrix. Nonetheless, a controlled and sustained drug release profile can be observed from a nanospanlastic ophthalmic gel in contrast to both pure azithromycin aqueous solution and nanospanlastic suspension.

Table 13. In Vitro Drug Release Comparison

Time (h)	Pure AZM solution (%)	Nanospanlastics (%)	Nanospanlastic gel (%)
1	42.3 ± 1.8	28.6 ± 1.4	18.2 ± 1.1
2	65.7 ± 2.1	45.9 ± 1.9	32.8 ± 1.6
4	88.4 ± 2.6	68.3 ± 2.3	54.6 ± 2.0
8	–	85.2 ± 2.7	72.9 ± 2.4

Table 14 presents the ex vivo corneal permeation parameters of optimized acetazolamide-loaded nanospanlastic gel. The values of Jss were 11.65 $\mu g \cdot cm^{-2} \cdot h^{-1}$ in Region I, 3–5 h, and 11.02 $\mu g \cdot cm^{-2} \cdot h^{-1}$ in Region II, 1–8 h, indicating efficient and sustained transcorneal permeation of acetazolamide from the formulation. High correlation coefficients, R^2 = 0.9999 and 0.9978, showed excellent linearity of permeation profiles in both regions, which further indicated consistent drug transport across the corneal tissue during the time course. The flux values being comparable at different time intervals prove the controlled and steady release of the nanospanlastic gel system. These results evidence the capability of the elastic nanospanlastic vesicles, coupled with the mucoadhesive gel matrix, to enhance corneal permeation, maintaining the sustained delivery of drugs for ocular therapy in glaucoma management.

Table 14. Ex Vivo Corneal Permeation Parameters

Linear Region	Time Interval (h)	Flux Jss ($\mu g \cdot cm^{-2} \cdot h^{-1}$)	R^2
Region I	3–5	11.65	0.9999
Region II	1–8	11.02	0.9978

Table 15 displays the stability evaluation of optimized acetazolamide-loaded nanospanlastic gel prepared for a period of three months. According to these physicochemical parameters, it can be depicted that a gradual increase in particle size has been observed from 182.4 ± 4.1 nm to 189.3 ± 5.8 nm with respect to initial and final time points. Overall, it has been realized from Table 15 that slight changes have been observed in PDI; hence, it has been noted to be within acceptable levels up to three months. In addition, slight decreases in zeta potential have been observed with respect to initial and final points; however, these have been within acceptable ranges for stability studies. Overall, slight decreases in entrapment efficiency from 86.3 ± 1.4 to 83.9 ± 2.1 and drug content from 99.1 ± 0.8 to 96.9 ± 1.3 have been noted to be within acceptable levels. Therefore, the constant rate has been observed in terms of stability evaluation of developed acetazolamide-loaded nanospanlastic gel, confirming its stability with respect to physical and chemical stability studies.

Table 15. Stability Study of Optimized Nanospanlastic Gel

Time (Months)	Particle Size (nm)	PDI	Zeta Potential (mV)	EE (%)	Drug Content (%)
0	182.4 ± 4.1	0.228	−28.6 ± 1.2	86.3 ± 1.4	99.1 ± 0.8
1	184.1 ± 4.5	0.231	−27.9 ± 1.4	85.6 ± 1.6	98.4 ± 0.9
2	186.8 ± 5.2	0.235	−27.2 ± 1.6	84.8 ± 1.9	97.6 ± 1.1
3	189.3 ± 5.8	0.241	−26.8 ± 1.7	83.9 ± 2.1	9 ± 1.3

Discussion

The present investigation was successfully demonstrated the effectiveness of the acetazolamide-containing nanospanlastic ocular gel, thereby highlighting the prospects of this advanced form of drug delivery system in the management of cases of glaucoma. The outcome of the present study was also in agreement, as well as supported, by the previous research work done on nanovesicular ocular delivery systems.

Preformulation evaluation demonstrated that acetazolamide meets pharmacopoeial requirements with respect to organoleptic properties, melting points, as well as moisture content. Similar findings have been mentioned by Sethi and Myrdal (2016), which emphasize the importance of the physicochemical purity and thermal stability of acetazolamide to ensure its successful integration into lipid-based carrier particles [13]. Similar findings were observed in terms of the low melting range in the present study, as mentioned by Khalil et al. (2017), which highlighted the importance of thermodynamic stability in preventing degradation of a drug during the preparation of nanovesicles by sonication or mild heating [14].

The solubility studies revealed low solubility of acetazolamide in water, which is consistent with its classification as a poorly soluble drug. According to the findings of Shukr and Aboelwafa (2022), sulfonamide compounds have higher solubility in physiological and buffered environments following incomplete ionization of the compound [15]. A similar trend is observed in simulated tears and buffered solutions as described in this study. The low solubility of the drug further underscores the importance of nano-based drug delivery systems. Similar problems and solutions have also been addressed by Rasheed et al. (2021) on methazolamide-filled nanovesicles [16].

The optimized nanospanlastic preparation exhibited a vesicle size of ~155 nm with a small distribution and sufficient surface charge, which is ideal for ocular drug delivery. Manconi et al. (2019) reported that vesicle sizes less than 200 nm improve corneal permeation and the vesicles) exhibit enhanced stability, a fact that is largely supported by the results of the present study, considering the vesicle size achieved in this study [17]. In addition, Sahin et al. (2025) reported that elastic nature of spanlastic vesicles improves the deformability of the vesicles [18], thus facilitating the transport of the vesicles through the corneal tight junctions easily.

The high Entrapment efficiency of the present study can be correlated in terms of values reported by Gupta et al. (2018), who showed that their Span-based nanovesicles exhibit high drug retention capability via hydrophobic interactions in the bilayer [19]. Abdelrahman and Abdelbary (2021) reported that the enhancement in EE of nanovesicles directly translates into the sustained release of the drug and hence more duration of activity when incorporated in eye preparations [20].

The morphological analysis using the SEM showed successful vesicles formed in spherical shapes with smooth surfaces. These observations agree with the findings in the study done by Rasheed et al. (2021) [16], where the spherical shape is essential in the maintenance of the formulation's stability in the nanospanlastic system, as noted in the study by Muzammil et al. (2022) [21].

Spectroscopic compatibility studies like UV spectroscopy and FTIR spectroscopy validate the lack of interaction between acetazolamide and the vehicle's formulation agents. Similar results have also been reported by Abdelkader et al. (2012), and the load's chemical integrity, like the case of the drug within the lipid vehicles, is essential to ensure therapeutic and stability attributes [22].

The nanospanlastic ocular gel possesses appropriate physicochemical properties such as physiological pH, appropriate viscosity, and appropriate spreadability, showing compatibility and patient acceptability for ocular application. Ophthalmic gels with appropriate viscosity and spreadability enable an increased precorneal residence time without producing discomfort, and these results are consistent with the characteristics of the developed ocular formulation, as reported by Pal Kaur and Kanwar (2002) [23].

In vitro drug release kinetics showed sustained drug release characteristics by the nanospanlastic gel, unlike the pure solution form, which can be explained on the basis of the combined mechanism of diffusion. Similar sustained release characteristics were also described by other authors, such as Abdelrahman et al. (2021), who described controlled release characteristics due to vesicle encapsulation, as reported by Rasheed et al. (2021), based on increased solution viscosity [16]. There was no bursting release, indicating the safety aspects of the formulation.

Ex vivo corneal permeation results showed enhanced and steady transcorneal flux consistent with study of Patel et al. (2013) [24-27] and Sahin et al. (2025) [18], who showed that elastic nanovesicles can significantly enhance corneal permeation through deformation and thus can penetrate corneal epithelial barriers. The steady-state flux values obtained in the present study depict efficient, controlled transport for maintaining the desired therapeutic intraocular pressure control.

Stability studies carried out for a period of three months showed marginal variation in particle size, entrapment efficiency, and drug content, indicating the robustness of the formulation. The observed stability profile corroborates findings by Abdelrahman and Abdelbary (2021) and Manconi et al. (2019), who demonstrated that nanovesicular ocular gels remain physically and chemically stable under recommended storage conditions [20,17].

Overall, the present study compared to previously reported nanovesicular ocular delivery systems, the developed acetazolamide-loaded nanospanlastic gel in the present study exhibited a comparable or superior performance regarding particle size, entrapment efficiency, sustained release, corneal permeation, and stability. These findings together reinforce the potential of nanospanlastic gels as an effective, patient-friendly, clinically promising approach for the therapy of glaucoma.

Conclusion

The present investigation successfully developed and characterized an acetazolamide-loaded nanospanlastic ocular gel intended to enhance ocular bioavailability and achieve sustained drug delivery for effective management of glaucoma. The optimized formulation provided nanosized, stable, and uniformly distributed elastic vesicles characterized by high drug entrapment efficiency and good physicochemical compatibility. Its incorporation into an ocular gel yielded a formulation with physiological pH, appropriate viscosity, and enhanced precorneal residence time. In vitro and ex vivo studies demonstrated sustained drug release and improved corneal permeation compared to conventional formulations, suggesting the possibility of decreasing dosing frequency and improving patient compliance. Stability studies further ascertained the stability of the formulation upon storage. Thus, Nanospanlastic-based ocular gel represents a promising, patient-friendly drug delivery platform for the therapy of glaucoma and deserves further in vivo and clinical investigations.

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