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Formulation And Evaluation Of Opthalmic Leciplex

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Abstract
Background: *Brinzolamide is a carbonic anhydrase inhibitor used in the treatment of glaucoma, but its ocular bioavailability is limited due to rapid precorneal clearance. Enhancing its retention and controlled release in the ocular cavity may significantly improve therapeutic outcomes.*
Objective: *To develop and optimize a phospholipid-based cationic lecithin complex (leciplex) incorporating brinzolamide to improve ocular retention, bioavailability, and therapeutic efficacy.*
Methods: *Leciplex formulations were prepared via a simple one-step process using phospholipids and cationic surfactants to promote mucoadhesion through electrostatic interaction with mucin. FTIR spectroscopy confirmed drug–excipient compatibility, while DSC and XRD were used to evaluate drug encapsulation and physical state. A Box–Behnken Design (BBD) was applied for optimization using Derringer-Suich desirability function. The optimized batch (F2) was characterized for particle size, PDI, encapsulation efficiency (%EE), drug loading (%DL), and stability. In vitro drug release and stability studies under accelerated conditions were also conducted.*
FTIR revealed no significant interactions between brinzolamide and excipients. DSC and XRD analyses indicated successful encapsulation and conversion of the drug to an amorphous form. The optimized batch (F2) achieved a desirability score of 0.998 and exhibited ideal particle size, narrow PDI, and high %EE and %DL. In vitro studies showed sustained release of 95.4% ± 1.1% over 8 hours, following first-order kinetics, compared to 93.8% ± 2.1% in 5 hours for the pure drug solution. The formulation remained stable with minimal variation in physicochemical properties under accelerated conditions.
Conclusion: *The brinzolamide-loaded cationic lecithin complex developed in this study demonstrated enhanced mucoadhesion, controlled release, and physical stability, suggesting strong potential for improved ocular delivery in glaucoma treatment. Further in vivo and clinical studies are warranted to confirm its therapeutic benefits.*
Keywords: *Brinzolamide, Leciplex, Timolol, Ocular drug delivery, Mucoadhesion, Box–Behnken Design, Controlled release, Glaucoma*

INTRODUCTION

The human eye, a highly sensitive and complex organ, plays a critical role in visual perception and overall sensory integration. Due to its anatomical and physiological barriers, effective ocular drug delivery remains a significant challenge, particularly for conditions affecting the posterior segment such as age-related macular degeneration, diabetic retinopathy, and glaucoma. These barriers— tear drainage, blinking, low conjunctival volume, and efflux transporters—limit drug absorption, with less than 5% of topically administered drugs reaching intraocular tissues.

Despite the widespread use of topical eye drops, which account for nearly 90% of ophthalmic formulations, conventional delivery systems often suffer from poor bioavailability, limited retention time, and patient discomfort. This has prompted growing interest in advanced drug delivery technologies, particularly those leveraging nanotechnology. Nanocarriers such as liposomes, nanosuspensions, leciplexes, and nanoemulsions offer advantages including enhanced corneal penetration, mucoadhesion, sustained drug release, and reduced dosing frequency. Notably, FDA-approved formulations such as Restasis® and DuraSite® have demonstrated the clinical viability of such approaches.

Among these, cationic lipid-based systems like leciplex have gained attention for their ability to interact electrostatically with negatively charged mucins on the ocular surface, thereby improving drug residence time. However, formulation stability and sterility remain critical challenges that must be addressed to ensure reproducibility and clinical translation. This study explores the development and optimization of a cationic lecithin complex incorporating brinzolamide, aiming to enhance ocular retention, therapeutic efficacy, and

bioavailability in glaucoma treatment.

2. MATERIALS AND METHODS

2.1 Materials

All chemicals and reagents used in this study were of analytical grade and procured from reliable commercial sources. Brinzolamide and Timolol, the active pharmaceutical ingredients, were obtained from MAXXIS Laboratories, Mumbai. Soy phosphatidylcholine, a key phospholipid component for lecithin complex formulation, was supplied by Saipro Biotech Pvt. Ltd., Pune. The cationic surfactant dimethyldioctadecylammonium bromide (DDAB), essential for imparting a positive charge to the lecithin complex, was procured from Antares Chem Pvt. Ltd., Pune. Transcutol® P, employed as a solubilizer and penetration enhancer, was provided by Kundan Distributors Pvt. Ltd., Pune, who also supplied essential excipients including sodium chloride, calcium chloride, sodium bicarbonate, and sodium hydroxide—used in the preparation and pH adjustment of various formulation components. Simulated Tear Fluid (STF), required for in vitro evaluations to mimic ocular conditions, was obtained from Spectra Vision Care, Pune. All materials were used as received without further purification, and were selected based on their compatibility and relevance to ophthalmic formulation development.

2.2 Preparation of Opthalmic Leciplex

Brinzolamide (BRZ)-loaded Leciplex formulations were developed using a simple, one-step fabrication method. This process involved the combination of negatively charged lecithin-based phospholipids with cationic surfactants in the presence of biocompatible solvents to enhance the delivery of the hydrophobic drug. In the formulation, soy-derived phosphatidylcholine (SPC) served as the phospholipid, while didodecyldimethylammonium bromide (DDAB), a double-chain cationic surfactant, was used at varying concentrations. Both components were accurately weighed and transferred into a beaker. A biocompatible solvent, Transcutol® P, was added to dissolve the lipid phase, and the mixture was heated to 70 °C in a temperature-controlled water bath.

Once the lipids were fully dissolved, the weighed amount of BRZ was incorporated into the solution, and heating continued until a clear, homogenous solution was obtained. Simultaneously, double-distilled water—also maintained at 70 °C—was added all at once to the mixture under continuous magnetic stirring at approximately 1300 rpm. This facilitated homogenization and led to the formation of BRZ Loaded Leciplex nanoparticles.

2.3Optimization Using Experimental Design

Following preliminary trial-and-error experiments, various lipid-to-surfactant ratios were identified for further evaluation based on critical formulation parameters. To systematically investigate the effects of formulation components and optimize the drug delivery system, a statistical approach using Response Surface Methodology (RSM) was employed. RSM integrates mathematical and statistical tools to model and analyze complex formulation variables, offering a robust framework for process optimization. Its applicability is well-documented in pharmaceutical formulation development due to its ability to construct empirical models from experimental data.

Among the various RSM approaches, the Box–Behnken Design (BBD) was selected for its efficiency in requiring fewer experimental runs and avoiding extreme combinations of input variables. This design is particularly effective in analyzing linear, quadratic, and interaction effects, facilitating the identification of optimal conditions for novel formulations (Wang et al., 2014).

In this study, a 3-factor, 3-level BBD was implemented using Design-Expert® software (Version 12, Stat-Ease Inc., Minneapolis, USA). A total of 17 experimental runs were conducted to evaluate the influence of three independent variables: phospholipid concentration (A), surfactant concentration (B), and drug amount (C), each tested at low, medium, and high levels determined from preliminary studies. The dependent variables, or responses, included particle size (R1),

polydispersity index (PDI, R2), entrapment efficiency (EE, R3), and drug loading (R4) for the brinzolamide-loaded leciplex formulation.

Table 1: Composition of Brinzolamide, Timolol Opthalmic Leciplex

		Actual value variables	Coded value variables

Run	Batch no.	Factor: A Phospho--lipid (mg)	Factor: B Surfac--tant (mg)	Factor: C: Drug (mg)	Factor: A Phospho--lipid (mg)	Factor: B Surfac--tant (mg)	Factor: C: Drug (mg)
1	F1	186	92.5	30	0	0	0
2	F2	217	92.5	40	0	0	0
3	F3	186	111	40	0	+1	-1
4	F4	155	111	30	0	+1	+1
5	F5	155	92.5	40	+1	+1	0
6	F6	217	129.5	40	-1	+1	0
7	F7	186	92.5	50	+1	-1	0
8	F8	217	111	50	0	0	0
9	F9	186	111	30	0	-1	+1
10	F10	217	111	40	-1	-1	0
11	F11	217	111	40	-1	0	+1
12	F12	186	129.5	30	+1	0	-1
13	F13	186	111	50	+1	0	+1

3. RESULTS

3.1Optimization of Phytosome Formulation

To achieve the most optimized batch, the software proposed a total of five solutions based on desirability values close to one. Desirability is defined as the geometric mean of individual desirability scores for PS, PDI, %EE, and %DL. These desirability scores range from 0 to 1, reflecting how close a response is to its ideal value. A higher desirability score indicates that the predicted responses align with the expected outcomes. Based on the desirability criteria, batch F2 was chosen as the optimized batch.

3.2 Physicochemical Characterization Particle Size

The particle size of optimized batch (F2) was found to 228.60± 2.4 nm and polydispersity index to be 0.09.

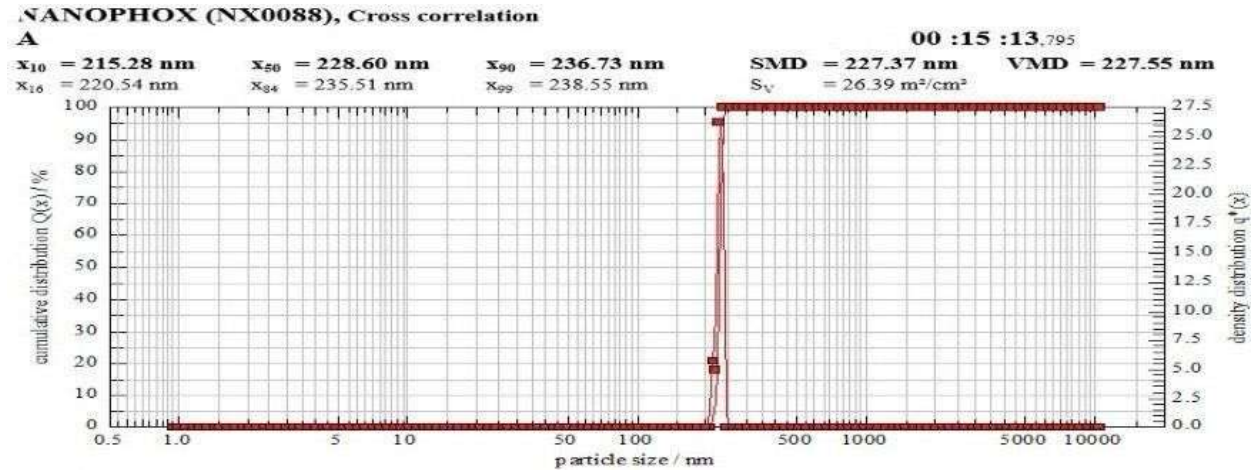

Figure 1: Particle Size of Optimized batch

Zeta Potential

The BRZ-loaded leciplex exhibited a positive zeta potential, attributed to the presence of the double-chain cationic surfactant (DDAB), which suggests good colloidal stability. The preference for a positive surface charge in these formulations is intended to enhance electrostatic interactions between the cationic leciplex and the negatively charged sialic acid residues present in corneal mucins. Among the tested batches, the optimized formulation (F2) demonstrated the highest zeta potential value (42.7 ± 0.80), as illustrated in Figure 3.13, confirming the stabilizing role of the cationic surfactant DDAB.

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Entrapment Efficiency (EE)

The optimized Brinzolamide leciplex shows 98% ±1.3 entrapment efficiency along with 32.05% ±0.5 drug loading capacity.

FTIR Analysis

The infrared (IR) spectrum of Brinzolamide was obtained using FTIR spectroscopy. The resulting IR spectrum is illustrated in Figure 3.1, and the characteristic functional groups identified are listed in Table 2.

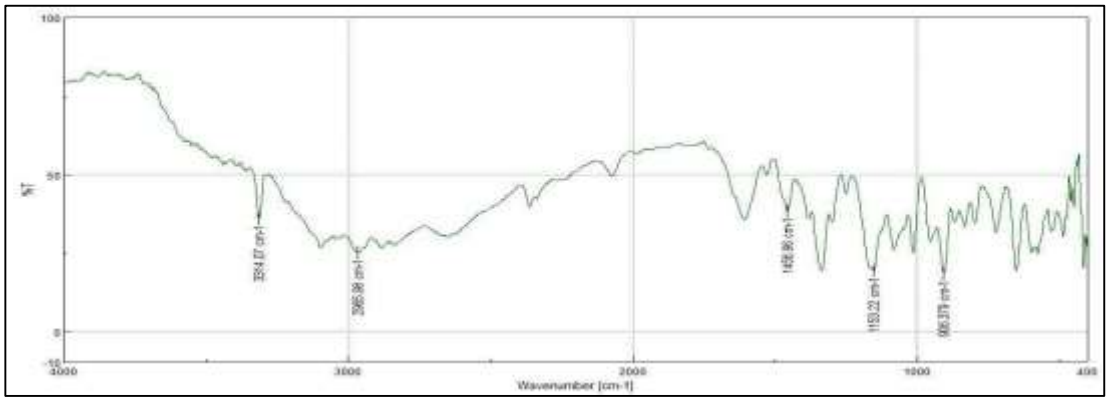

Figure 2: FTIR of Brinzolamide

Table No.2: IR frequencies of Brinzolamide functional group

Functional Group	Reported IR frequencies(cm^{-1})	Observed IR frequencies(cm^{-1})
-NH stretching sulfonamide	3400 -2900	3314.407
Aliphatic C-H stretching	3000-2800	2955.99
CH_3 deformation	1470-1440	1456.96
Symmetric stretch O=S=O	1173-1155	1153.22
S-N stretching of sulfonamide	904	906.379

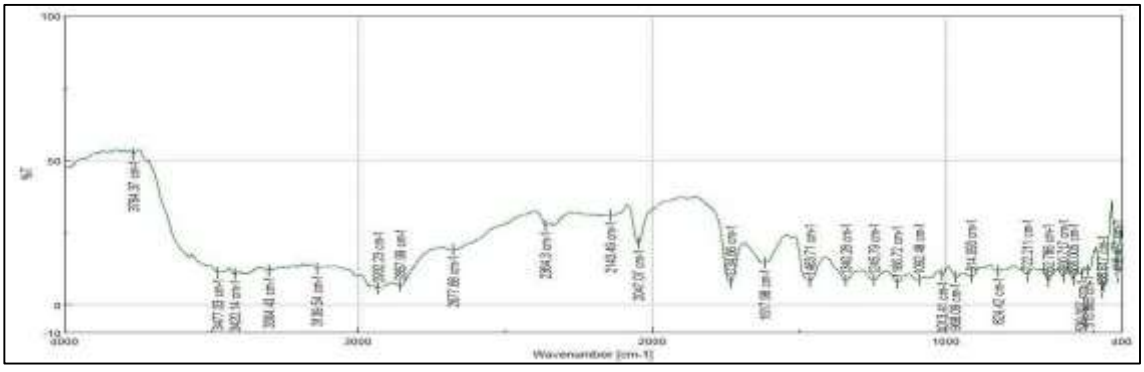

Figure no.3 (a) FTIR of Physical Mixture (1:1)

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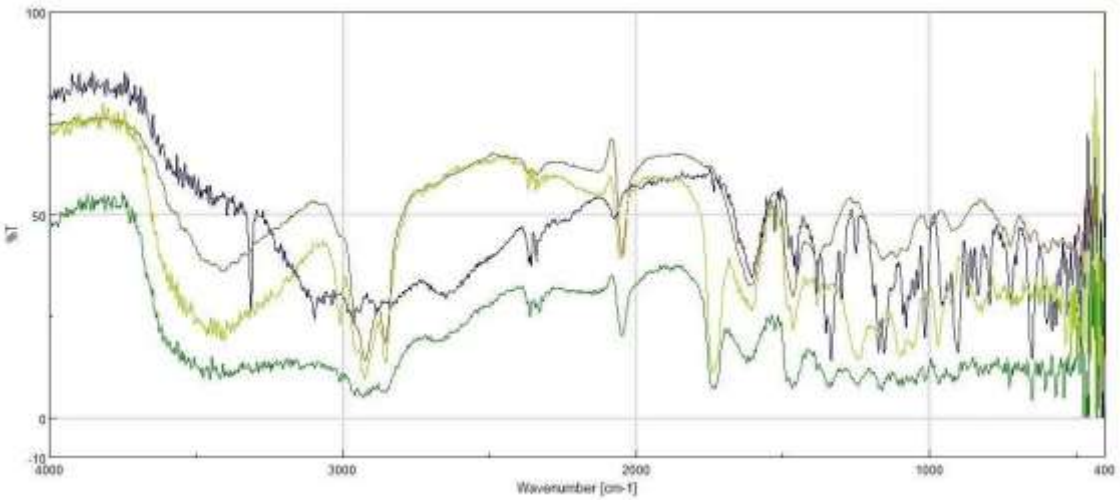

Fig. no.4 (b) FTIR Overlay of (A) BRZ, (B) SPC, (C) DDAB (D) Physical mixture

DSC Analysis

Differential Scanning Calorimetry (DSC) was employed to analyze the thermal properties of Brinzolamide. A distinct endothermic peak for the pure drug was observed at 133.16°C.

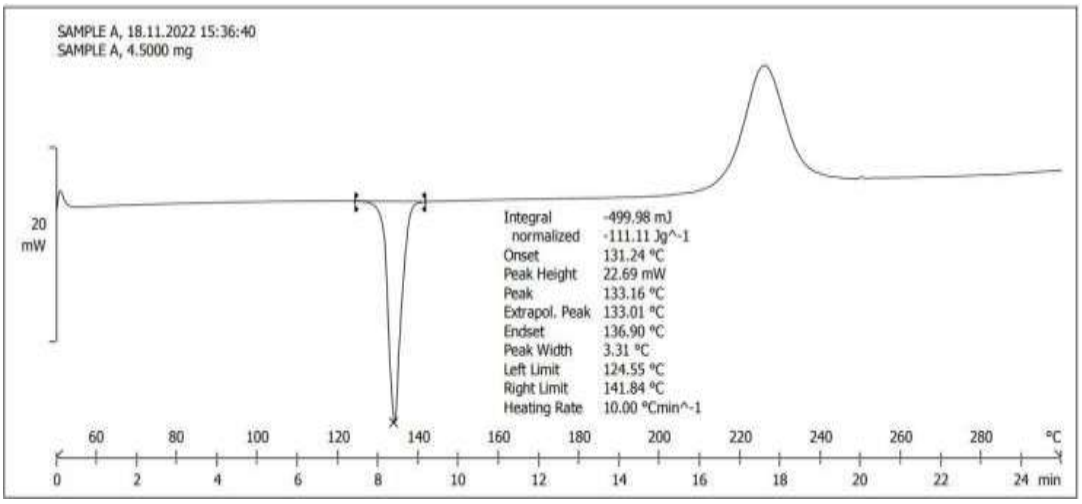

Fig no. 5 DSC thermogram of Brinzolamide

3.3In Vitro Drug Release

An in vitro drug release study was performed to evaluate the release profile of Brinzolamide from the lipid matrix formulated for ocular delivery, specifically for batch F2. The percentage of cumulative drug release is presented in Figure 3.17. The in vitro release profile of the optimized formulation, assessed using a dialysis membrane (Table 3), demonstrated an initial burst release within the first 2 hours. Subsequently, the marketed Brinzolamide formulation exhibited 93.8% drug release within 6 hours. In contrast, the Brinzolamide-loaded Leciplex formulation showed a sustained release, achieving 98.45% drug release over a period of 8 hours from the lipid matrix.

Table no.3 *In vitro* drug release study for BRZ Leciplex and BRZ marketed.

Time (hr.)	BRZ Marketed (% Cumulative release)	BRZ Leciplex (% Cumulative release)
0	0	0
1	38.12±2.1	21±2.7
2	49.65±3.4	36.25±3.4
3	73.36±2.5	46.2±3.5

4	85.2±0.9	54.25±4.2
5	90.1±1.8	67.65±3.4

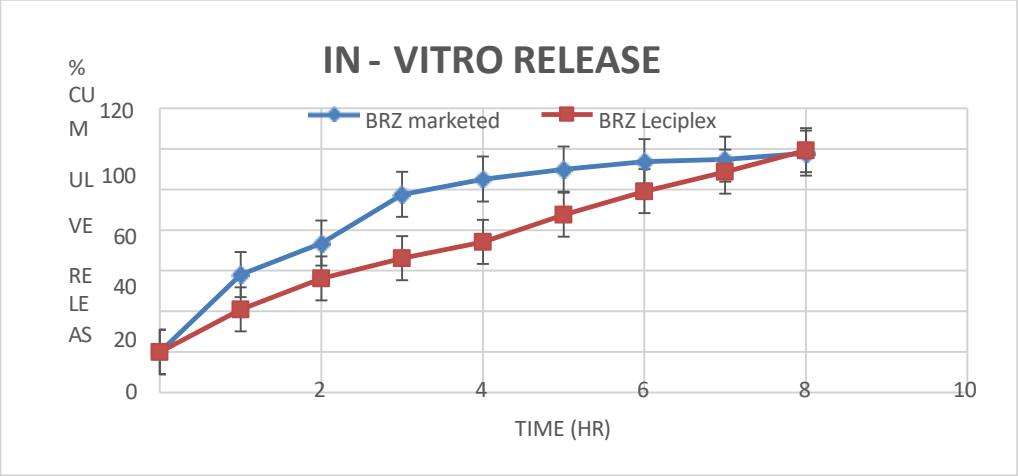

Fig. no.6 Cumulative % drug release study

3.4 Stability Studies

A three-month stability study was conducted on the optimized formulation batch F2 to evaluate its resistance to drug degradation and formulation instability. The study monitored critical parameters, including drug particle size, encapsulation efficiency (EE), and polydispersity index (PDI), to assess the formulation's stability over time. The results indicated that these parameters remained consistent throughout the testing period, suggesting that the formulation maintained its structural integrity and performance characteristics. Additionally, the physical appearance of the formulation did not exhibit any significant changes, further supporting its stability under the test conditions. These findings align with established guidelines for stability testing, confirming that the optimized batch F2 retains its intended quality attributes over the duration of the study.

Table no.4 Accelerated stability study

Months	Storage condition	Appearance	Particle size (nm)	PDI	% EE	% DL
1	$40^0C±2^0C/75\%$ RH±5%RH	No change	270.17	0.2	96.25	31.32
2			252.25	0.3	94.14	28.71
3			224.63	0.3	88.36	32.91

CONCLUSION

A phospholipid-based cationic lecithin complex (leciplex) was successfully developed for ocular delivery of brinzolamide using a simple one-step fabrication method. The optimized formulation (F2) exhibited favorable physicochemical properties, including appropriate particle size, low polydispersity index (PDI), and high encapsulation efficiency (%EE), indicating efficient drug incorporation. Stability studies under accelerated conditions confirmed the formulation's robustness, showing minimal variations in particle size, PDI, %EE, and drug loading (%DL), thereby demonstrating its long-term stability. In vitro release studies revealed a sustained drug release of 95.4% ± 1.1% over 8 hours from F2, compared to 93.8% ± 2.1% over 5 hours from the pure drug solution. The release kinetics followed a first-order model, indicating concentration- dependent drug release. Formulation optimization was achieved using Box–Behnken Design (BBD), allowing effective modeling of factor–response relationships with reduced experimental runs. Differential Scanning Calorimetry (DSC) analysis confirmed the absence of the drug's characteristic melting peak, suggesting molecular dispersion or amorphization. X-ray Diffraction (XRD) further supported this, showing a broad halo pattern without distinct peaks, indicative of an amorphous state, which can enhance solubility and ocular bioavailability. Overall, the developed brinzolamide-loaded leciplex demonstrates significant potential as an effective ocular drug delivery system for the treatment of glaucoma.

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