

RESEARCH ARTICLE

Preparation and Evaluation of Rebamipide Ophthalmic Nanosuspension for Dry Eye Therapy

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ABSTRACT

Dry eye syndrome poses a therapeutic challenge due to the limited residence time of traditional treatments on the ocular surface. The commercially available rebamipide (REB) ophthalmic suspension, though approved for clinical use in dry eye patients, suffers from rapid drainage through the nasolacrimal duct, limiting its effectiveness. In this study, rebamipide nanosuspension was successfully developed by solvent-diffusion methodology and investigated for measurement of particle size, shape, surface charge, fourier-transform infrared (FTIR), differential scanning calorimetry (DSC), percentage entrapment efficiency, dissolution studies and release kinetics, *ex-vivo* permeability study. Results demonstrate that the optimized formulation has 196 nm particle size, surface charge of +32.5 mV, and low polydispersity. FTIR studies revealed that the drug exhibits compatibility with the excipients integrated into the formulation. DSC and X-ray diffraction (XRD) analysis revealed a decrease in the crystalline nature of the drug. Transmission electron microscope (TEM) findings indicate that rebamipide nanosuspension possesses a spherical shape, and *in-vitro* studies demonstrate a slower and sustained release of REB. *Ex-vivo* studies performed on excised goat corneas demonstrated significantly improved drug permeation compared to the suspension without inducing corneal damage.

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INTRODUCTION

Dry eye is a significant public health issue that affects the tear film and can result from a lack of tears or too much tear evaporation. It can lead to damage on the surface of the eye and is accompanied by various symptoms such as eye discomfort, fatigue, and visual disturbances, potentially impacting daily activities.¹ Moreover, individuals with dry eye are more susceptible to severe eye infections like bacterial keratitis and face complications after laser refractive surgery.² Dry eye management involves various approaches, including dietary modifications, educating the patient, environmental adjustments, using lubricating drops, employing punctual plugs, medicaments, and surgery options. The selection of a treatment approach is contingent on the underlying cause and the extent of the condition.^{3,4}

Rebamipide is slightly acidic drug, water-insoluble (Figure 1) and belongs to class IV in the Biopharmaceutical Classification System (BCS). Rebamipide has various pharmacological effects such as increasing mucus production and secretion, goblet cell density, promoting wound healing, and exhibiting cytoprotective and anti-inflammatory properties. Rebamipide has been marketed in Japan as Mucosta® 2% ophthalmic suspension since 2012 for its effectiveness in treating dry eyes.⁵

Aqueous ocular drops represent the most prevalent and frequently employed means of administering ophthalmic medications. However, a significant portion of the applied dose is lost before it can reach the desired ocular tissues. This is due to factors like drainage and blinking. To achieve effective drug concentrations in the eye, frequent application of these drops is necessary. To improve the ocular retention of aqueous drops on the surface and improve drug availability, researchers have explored using additives like viscosity modifiers and bioadhesives.⁶ Nanoparticles, due to their very small size, do not cause irritation in the eye and stay longer in the cul-de-sac, slowly releasing the drug. Previous studies with drugs like diclofenac, aceclofenac, and itraconazole have shown that nanoparticle formulations result in better penetration and bioavailability of the drug in the eye.

This study aims to develop and assess the prepared rebamipide nanosuspensions intended for managing dry eye syndrome. This drug delivery system utilizes chitosan as a key component, combining the benefits of rebamipide mucin secretion enhancement and the lubricating properties of chitosan. Rebamipide nanosuspension was fabricated using the solvent diffusion technique with probe sonication.

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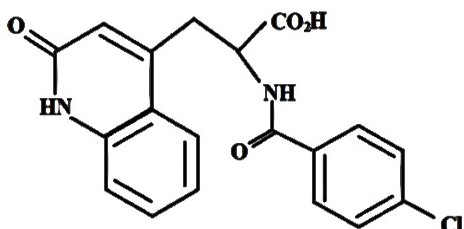


Figure 1: Structure of rebamipide

MATERIAL AND METHODS

Materials

Rebamipide (REB) drug was procured from Shiva Chemical, Delhi. Poloxamer-188, water-soluble chitosan, and hydroxypropyl methylcellulose were purchased from Sigma Aldrich, Delhi. Analytical-grade chemicals and reagents were employed in this study.

Methods

Preparing rebamipide nanosuspension

The preparation of the REB nanosuspension involved a series of steps. Firstly, the required quantity of the drug was dispersed in a phosphate buffer (pH 7.4) with continuous stirring. Then, this prepared solution was slowly added to another solution, which contained water-soluble chitosan and finally, HPMC and Poloxamer-188 were introduced using a syringe with 24G needle. The resultant solution was subjected to a homogenization procedure utilizing a high speed homogenizer operating at 6000 rpm for 20 to 25 minutes. Subsequently, the mixture is subjected to probe sonication for a period lasting between 8 to 15 minutes to further enhance the quality of the nanosuspension.⁷

Nanoparticle size, surface charge, and PDI

The particle size and surface charge of rebamipide nanosuspension were determined using Zetasizer Nano instrument (Malvern, UK).⁸

Fourier transform infrared

IR spectra of the samples were recorded using a Shimadzu, Fourier transform infrared (FTIR) spectrophotometer (Tokyo, Japan). The spectra were captured within the range of 4000 to 400 cm^{-1} .⁹

Differential scanning calorimetry

For differential scanning calorimetry (DSC) evaluation, TA instrument (Shimadzu, Japan) was utilized for thermal analysis. Approximately 10 mg of the sample was enclosed in an aluminum pan, and then it was subjected to a controlled heating process in the temperature range of 10 to 380°C and the heating rate is 10°C/min.¹⁰

Morphology

The morphology of REB nanoscale particles within the formulation was examined by transmission electron microscope (TEM).

Entrapment efficiency of the nanoparticles

The optimized nanosuspension was ultracentrifuged for 10 to 20 minutes. After centrifugation was done, the clear aqueous phase was collected and analyzed using UV spectrophotometer.¹¹

In-vitro drug release analysis

Dissolution studies were conducted using 5.0 mL of freshly prepared nano-suspension placed inside a dialysis membrane, which was then immersed in (Simulated tear fluid (STF), pH 7.4) serving as the dissolution medium. This buffer was continuously stirred by a magnetic bar rotated at 50 rpm within the beaker. Samples (3 mL) were withdrawn at specified time periods and replaced by STF medium of equal volume. The collected samples were subsequently analyzed using UV spectroscopy (326 nm) and the release data obtained from these experiments were analyzed using various kinetic release models.¹²

Ex-vivo corneal permeation study

Drug permeation was studied using goat corneas in a Franz diffusion cell. The corneas were prepared and mounted with the epithelial side facing the donor compartment. The donor chamber contained nanosuspension, and the receptor chamber was filled with simulated tear fluid. Samples were withdrawn at specific intervals, fresh buffer was added, and the samples were analyzed for drug permeation using a UV-visible spectrophotometer.¹³

Analysis of corneal hydration levels (%HL)

To assess corneal hydration levels, the corneas were first submerged in methanol and were then subjected to drying at 90°C for an entire night, following the completion of the permeability studies. The change in corneal weight before and after drying was measured to determine the hydration level. Paired corneas were used to ensure consistent results and minimize biological variations during the permeation study.¹⁴

Stability studies of nano-formulation

The stability testing was conducted under two sets of conditions: 40°C/75% RH (accelerated temperature) and 25°C/60% RH (room temperature) conditions. At various time intervals, 0, 3, and 6 months, samples were collected for accelerated and room temperature conditions. These samples were then analyzed to assess the content of rebamipide over time.¹⁵

RESULT, DISCUSSION AND CONCLUSION

Nano-formulation: Particle Size, Surface Charge, and Particle Size Distribution

Figure 2 demonstrates the particle size (196 nm) of optimized preparation and a lower PDI value (0.188) containing 3.0% w/v of poloxamer 188, which served as a stabilizer, indicating excellent stability and uniform dispersion. All the nanoparticles in the study exhibited a positive charge, with the optimized formulation having the highest charge value of 35.50 which acts as mucoadhesive and beneficial for improving drug ocular

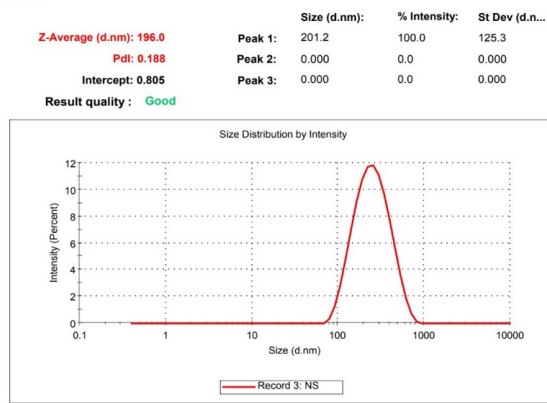


Figure 2: Nanoparticle size of optimized formulation

bioavailability, especially considering the negative charge of the corneal mucin layer. This suggests that these formulations exhibit the capability for improving drug delivery and efficacy in ophthalmic applications.

FTIR Analysis

Analysis of the FTIR spectra for pure REB and the optimized formulation revealed that there were no new chemical interactions or changes in the characteristic peaks (Figures 3 and 4). This suggests that the drug and the formulation materials do not chemically interact with each other. FTIR spectra of the pure REB showed sharp characteristic peaks: amines stretching (NH and CH₂) at 3630.4 to 3268.9 cm⁻¹, alkane stretching at 2105.9 cm⁻¹, C=O stretching of carboxylic acid and amide at 1722 and 1640 cm⁻¹, respectively and N-H bending of amide at 1535 cm⁻¹ were observed. This confirms that there were no chemical interactions altering the drug structure in the formulation.

DSC study

The DSC study provided insights into the thermal characteristics of the substances involved. Pure rebamipide exhibits an endothermic sharp peak at 306°C, which indicates its crystalline characteristics. Chitosan, Poloxamer-188, and hydroxypropyl methylcellulose exhibited broad endothermic peaks (82, 54, 88°C), respectively, suggesting their amorphous properties (Figure 5). DSC analysis of the rebamipide nanosuspension displayed several characteristics. It revealed an endothermic peak at 295°C, corresponding to rebamipide. Additionally, a broad and less pronounced endothermic response was observed, which is indicative of the presence of chitosan and HPMC in the formulation.

Shape Characterization and Entrapment Efficiency

TEM images of the rebamipide nanosuspension showed spherical nanoparticles with a smooth surface, as seen in Figure 6. These images confirmed that the optimized formulation contained nanoparticles that were consistently smaller than 300 nm in size, demonstrating their uniformity and small dimensions. The rebamipide nanosuspension effectively encapsulated 87% of the drug.

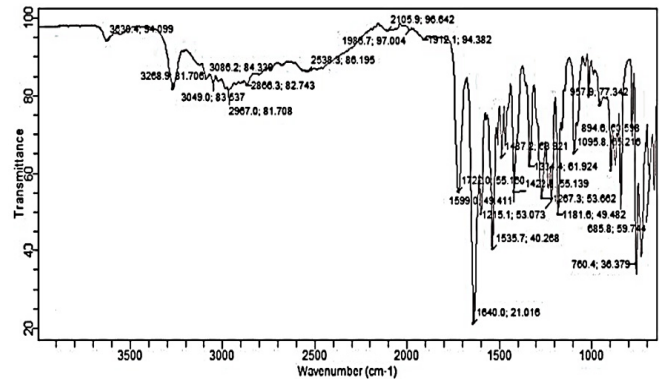


Figure 3: FTIR of rebamipide

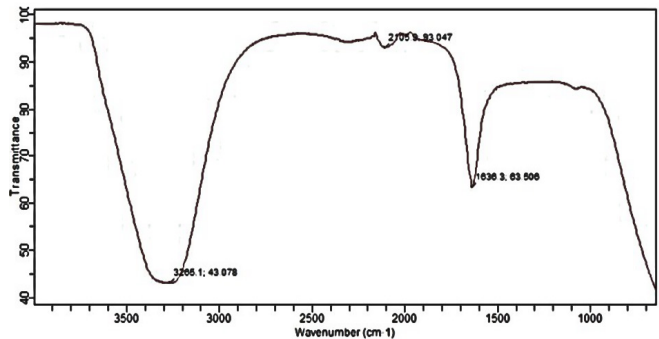


Figure 4: FTIR of optimized preparation

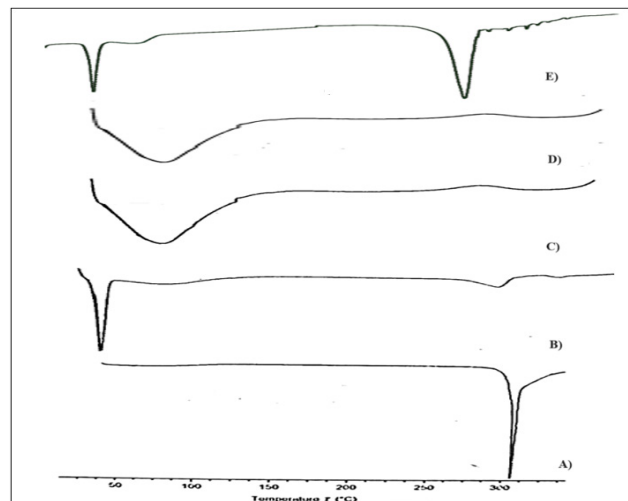


Figure 5: DSC A) Rebamipide, B) Poloxamer-188, C) Chitosan, D) Hydroxypropyl methylcellulose, and E) REB nanosuspension

In-vitro Release Profile

Drug release profiles demonstrated that optimized formulation prolonged the release of the rebamipide for up to 24 hours, as depicted in Figure 7. The optimized formulation, which consisted of 0.6% chitosan (water soluble) and HPMC in a 1:1 ratio and 3% Poloxamer 188 showed prolonged drug release. This effect was likely due to the muco-adhesive properties

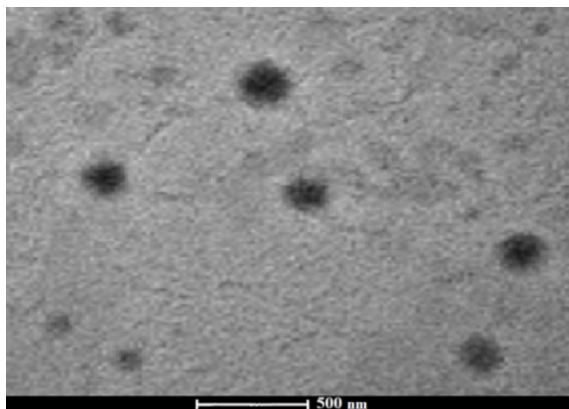


Figure 6: TEM images of nanoparticles

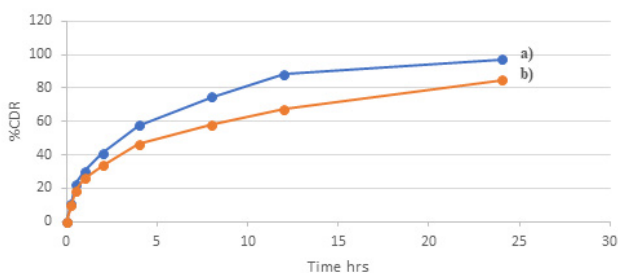


Figure 7: Dissolution profile of a) REB-suspension b) REB-nanosuspension

of the chitosan. Various kinetic models were employed to understand the mechanism of drug release from the nanoparticles. Among the different models, the drug release pattern of optimized formulation exhibited the best fit with the highest correlation coefficients when fitted to the Peppas-Korsmeyer kinetic model.

Ex-vivo Drug Permeation Profile

The optimized formulation exhibited enhanced drug release through chitosan adhesion to the corneal surface, resulting in improved drug permeation. Over an 8-hour period, the optimized formulation achieved a drug permeation rate of 86.70%, surpassing the performance of the traditional suspension. Significantly, the %HL remained within acceptable thresholds (80%), indicating that the optimized formulation enhanced drug delivery without causing harm to the corneal layers (Figure 8).

Stability Analysis

The stability study was carried out following ICH guidance for 6 months. During the study, a slight increment was noted in both the particle size and PDI under both storage conditions. It was determined that the prepared nanosuspension exhibited moderate stability. The presence of HPMC and Poloxamer-188 in the REB nanosuspension prevented crystal agglomeration by binding to the particle surfaces, improving their stability.

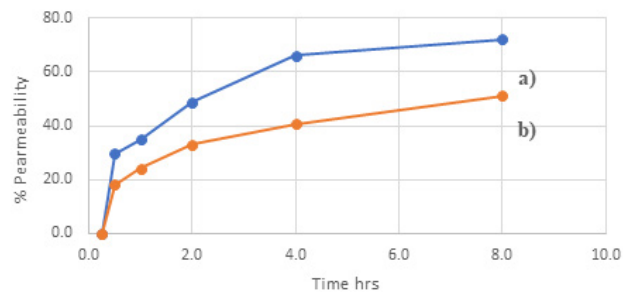


Figure 8: Ex-vivo Permeation study of (a) REB-NS and (b) REB suspension

CONCLUSION

In conclusion, our research suggests that the rebamipide nanosuspension we developed possesses the right particle size for ocular applications. These nanoparticles demonstrate extended drug release, increased tolerability, prolonged corneal retention, and improved stability. Therefore, these formulations have the potential to be valuable for treating dry eye conditions through topical application.

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