DEVELOPMENT AND CHARACTERIZATION OF REBAMIPIDE NANOPARTICULATE SYSTEM PREPARED BY SOLVENT DIFFUSION METHOD FOR OPHTHALMIC DELIVERY

Reena Tyagi[1]*, Vijay Bhalla[2], Prantika Mondal[3]

Abstract

Rebamipide nanosuspension was created through the utilization of the solvent diffusion method, and their properties were examined across several parameters. These assessments encompassed the determination of particle size and structure, zeta potential, encapsulation efficiency, in-vitro drug release behavior, and ex-vivo therapeutic effectiveness. The experiment revealed that altering the drug-to-polymer ratio had an impact on the particle size. The particulate formulation demonstrated a sustained drug release in-vitro profile, following the Korsmeyer-Peppas kinetic model, indicating controlled drug release over time. The drug release from the nanosuspension occurs through a combination of processes involving both dissolution and diffusion. This observation is supported by the experimental results obtained. The ideal formulation displayed a particle size of 196.0 nm and possessed a zeta potential of +32.5 mv. The particles were spherical in shape, and their uniformity was indicated by a low polydispersity index of 0.188. Differential Scanning Calorimetry and XRD studies showed a reduction in the crystalline nature of the drug. During ex-vivo transcorneal studies using excised goat corneas, it was observed that the drug's transcorneal permeation was higher when compared to the suspension of the drug. This increased permeation was achieved without causing any damage to the cornea. Additionally, stability studies indicated that the formulation remained stable under the specified conditions, further highlighting its potential as a drug delivery system.

Keywords: Ocular drug delivery, Rebamipide, Nanosuspension, Chitosan, Dry eye disease, Eye conditions

[1*]SGT College of Pharmacy, Department of Pharmaceutics, SGT University, Gurugram, Haryana, India.
[2]SGT College of Pharmacy, Department of Pharmaceutics, SGT University, Gurugram, Haryana, India.
[3]SGT College of Pharmacy, Department of Pharmaceutics, SGT University, Gurugram, Haryana, India.

***Corresponding Author :** Reena Tyagi
*****SGT College of Pharmacy, Department of Pharmaceutics, SGT University, Gurugram, Haryana, India.
E-mail: itsmeria.tyagi@gmail.com

DOI: - 10.31838/ecb/2023.12.s2.543

INTRODUCTION

Dry eye syndrome is a highly common ocular surface condition on a global scale. Studies have reported its prevalence to vary significantly, ranging from around 4.4% to as high as 50%, particularly among middle-aged and older populations worldwide. This wide range in prevalence underscores the significant impact of DED and its widespread occurrence among different demographic groups.[1] This condition presents with symptoms including discomfort, visual disturbances, and unstable tear film. Moreover, it has the potential to damage the eye's surface. This condition is frequently associated with an increase in the osmolarity (saltiness) of the tear film and inflammation of the tissues on the surface of the eye.[2] Dry eye disease (DED) is recognized for its substantial adverse effects on the quality of life, particularly in relation to visual aspects. It can result in difficulties in performing daily activities, further emphasizing the importance of diagnosing and managing this condition effectively.[3]

In the field of ophthalmology, rebamipide ophthalmic (2%) suspension has gained recognition for its effectiveness in treating dry eyes.[4] This ophthalmic suspension has been available on the Japanese market for the management of dry eye conditions since January 2012. It is considered a valuable therapeutic option for individuals experiencing dry eye symptoms in Japan.[5] Prior studies have shown that the application of topical rebamipide has the potential to enhance the quantity of goblet cells and induce the secretion of substances resembling mucin in the bulbar or ocular conjunctiva and caruncula lacrimalis of human subjects. This suggests a beneficial impact of rebamipide on the

ocular surface, potentially enhancing the mucus layer stability and the overall health of the eye.[6,7]

The use of ophthalmic drug application is the primary method for treating various eye conditions, and it is generally well-received by patients. However, in conventional formulations, a significant portion of the administered drug doesn't effectively reach its intended target within the eye. This is primarily due to dilution caused by the eye natural tearing mechanism and the drainage of the drug through the nasolacrimal duct, reducing the drug efficacy at the site of action.[8] As a result, patients often require frequent administration of eye drops to achieve the desired therapeutic effect. Therefore, there is a significant emphasis on the development of sustained drug delivery systems (DDS) in the field of ophthalmology. These systems aim to provide a consistent and prolonged release of medication, reducing the need for frequent applications and improving the overall convenience and effectiveness of eye treatments.[9]

To overcome the challenges mentioned earlier, the development of a drug delivery system like nanosuspensions offers significant advantages. Such a system can enhance the drug contact time with the eye and provide controlled or sustained release of the medication, addressing the limitations of traditional eye drops. Furthermore, this nanosuspension formulation can be administered in the form of eye drops, avoiding issues like blurred vision or irritation often associated with other formulations. Additionally, due to its sustained release characteristics, it would necessitate fewer administrations, leading to improved patient convenience and adherence to the treatment regimen. This represents a promising approach in the field of ophthalmology for more effective and patient-friendly management of eye conditions.[10]

In this research study, the focus was on the preparation of Rebamipide nanosuspension using the solvent diffusion method. The resulting nanosuspension underwent a series of characterizations, including Powder X-ray Diffractometry, Solubility test, Dissolution Analysis, Entrapment efficiency, DSC, Particle size analysis, and Zeta potential. Ex vivo permeability was also evaluated using goat cornea.

MATERIALS AND METHODS

Materials

Rebamipide raw material was obtained from Shiva Chemicals, Delhi. HPMC, Chitosan (water soluble), and Poloxamer 188, were obtained from Sigma Aldrich, Delhi. In the research study, all the ingredients employed were of analytical grade.

Methods:

Preparation of Rebamipide nanosuspension

The Rebamipide nanosuspension was prepared through the solvent diffusion method followed by the homogenization process. The drug was accurately measured and dissolved in phosphate buffer solution with a pH of 7.4 with continuous stirring. This drug solution was introduced slowly, drop by drop, into another solution containing water-soluble Chitosan, Hydroxypropyl Methylcellulose, and Poloxamer 188. The introduction was carried out using a syringe equipped with a 24-gauge needle. After combining the two solutions, the resulting mixture was homogenized vigorously using a high-speed homogenizer at 6000 rpm for a duration of 20 minutes. Following the homogenization step, magnetic stirring was employed for an additional 2 hours to further ensure uniform mixing. Subsequently, the solution underwent probe sonication at a frequency of 30 kHz for a period of 8-10 minutes.[11]

Particle size and PDI measurement

The analysis of particle size, zeta potential, and polydispersity index (PDI) was conducted using a Zetasizer Nano instrument from Malvern, UK, which was equipped with DTS (Dynamic Light Scattering) software.[12]

Fourier-transform infrared spectroscopy

The samples underwent Fourier-transform infrared (FT-IR) spectroscopy analysis using a FTIR spectrophotometer, covered the spectral range from 4000 to 400 cm^{-1}. To perform this analysis, the samples were prepared as KBr pellets, which involves mixing the sample with potassium bromide (KBr) to form a solid pellet.[13]

Differential scanning calorimetry

A certain quantity of either processed or unprocessed sample was loaded into an unsealed sample cell, while an empty sample cell was used as a reference. These cells were then subjected to thermal analysis using a differential scanning calorimeter. The thermal analysis involved heating the samples over a specified temperature range, starting from 10°C and going up to 350°C. The rate at which the temperature increased was set at 10°C per minute.[14]

X-ray powder diffraction

In this study, various samples including the REB bulk drug, blank excipients, and physical mixtures

were analyzed using an X-ray diffractometer. The X-ray diffraction pattern was obtained using a step scan model within a specified angle range, which was set between $3° < 2\theta < 45°$. The step size for data collection was set at 0.02°.[15]

Transmission electron microscopy

The morphological evaluation of the nano-sized particles in the prepared nanosuspension was conducted using transmission electron microscopy. The analysis was performed on a Tecnai T20, FEI, USA instrument at the desired magnification.[16]

Entrapment efficiency

In the determination of entrapment efficiency, a 10 mL sample of the freshly prepared nanosuspension was centrifuged at 1000 rpm for 10 minutes. After centrifugation, the supernatant was collected, and the unincorporated drug amount was quantified by measuring its absorbance at 326 nm using a UV-visible spectrophotometer. This measurement was essential for calculating the entrapment efficiency, a key parameter in evaluating how effectively the drug is encapsulated within the nanosuspension. [17]

In-vitro drug release study

In vitro studies, a 5 mL portion of freshly prepared nanosuspension was enclosed within a Dialysis membrane and submerged in a beaker filled with stimulated tear fluid buffer with a pH of 7.4. This buffer solution was used as the dissolution medium. The dissolution medium was kept in motion by a magnetic bar rotating at a speed of 50 rpm. At specified time intervals, samples of 3 mL in volume were extracted from the beaker and the same volume of fresh dissolution medium was introduced to maintain sink conditions. The samples that were withdrawn at various time points were subsequently analyzed using UV spectroscopy at a wavelength of 326 nm. The in-vitro release data underwent analysis using multiple kinetic models, which included the Zero order, First order, Peppas-Korsmeyer, Higuchi model, and Hixon-Crowell model.[18]

Ex vivo permeability studies

To investigate drug permeation through excised goat corneas, a modified Franz diffusion cell was utilized. Goat eyeballs were quickly obtained from a local slaughterhouse and the corneas were meticulously prepared for testing. These corneas were mounted onto the diffusion cell with the epithelial membrane facing the donor compartment. The receptor chamber contained STF buffer at pH 7.4, and the donor chamber held nanosuspension equivalent to a 20mg drug concentration. At specific time intervals, samples were withdrawn from the acceptor compartment and replaced with fresh STF buffer. These samples were then diluted, filtered, and analyzed using a UV-visible spectrophotometer to quantify drug permeation. The entire procedure was conducted in triplicate for accuracy.[12]

Corneal hydration studies

In corneal hydration studies, the corneas were immersed in methanol and subsequently dried at 90°C overnight following the permeation study. The level of corneal hydration was determined at the conclusion of the experiment by measuring the difference in corneal weights before and after drying. To ensure consistent results and avoid biological variations, paired corneas were employed in the permeation study.[18]

RESULTS AND DISCUSSION

Particle size, Zeta potential and Entrapment efficiency

The particle size of the formulations falls within a range of 145.5 to 200.3 nm, demonstrating a relatively consistent size distribution. Zeta potential values range from 10.1 to 32.5mV, with all nanoparticles exhibiting a positive charge and the polydispersity index (PDI) varies from 0.188 to 0.682. The optimized formulation has particle size 196.0 nm and lowest PDI value (0.188) as shown in figure 1 indicating good stability and uniform dispersion. Also, the positively charged nanoparticles are mucoadhesive, a beneficial property for increased drug ocular bioavailability, particularly since the corneal mucin layer has a negative charge. This suggests the potential of these formulations to enhance drug delivery and efficacy in ocular applications. Entrapment efficiency (%EE) of the Optimized rebamipide nanosuspension was determined to be 87%.

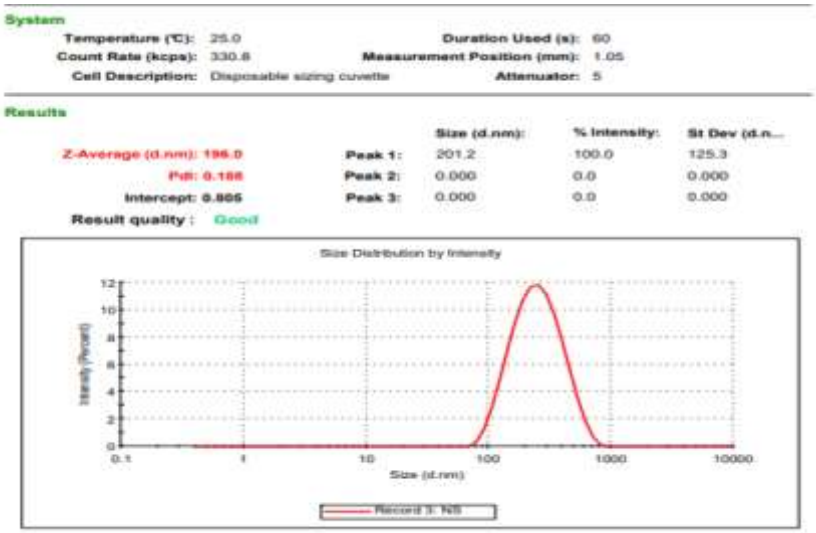

Figure 1: Particle size and PDI of the optimized formulation

FTIR spectroscopy analysis

FTIR spectra of pure Rebamipide, blank polymers, and Rebamipide optimized formulation were compared. No new peaks or disappearance of characteristic peaks were observed, indicating the absence of chemical interactions between the drug and polymer. The FTIR spectrum of the pure drug exhibited sharp peaks corresponding to amines stretching (N–H and CH2) vibrations at 3630.4 – 3268.9 cm−1, alkane stretching (–CH3, –CH2, and –CH) vibration at 2967.0 cm−1, C=O stretching at 1722.0 cm−1 (attributed to aldehydes), and C=O–NH stretching at 1640.0 cm−1. Furthermore, selective stretching vibrations at 1599.0 cm−1 and 1535.7 cm−1 for primary and secondary amines were observed.[19]

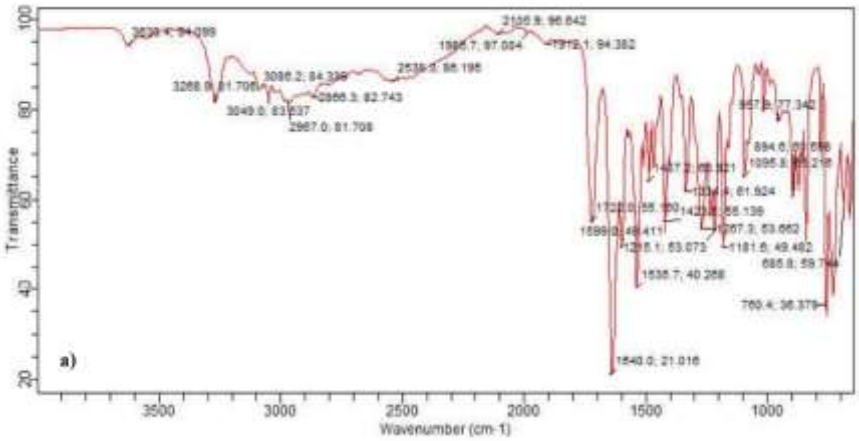

Figure 2: FT-IR spectrum of pure Rebamipide

DSC analysis

The thermal characteristics of pure Rebamipide drug and its physical mixture were assessed using differential scanning calorimetry. The DSC thermogram displayed a distinct endothermic peak at 306.01 °C, which corresponds to the melting point of Rebamipide (Figure 3). This sharp peak suggests the crystalline nature of the drug. The thermal analysis of chitosan and HPMC displayed a broad endotherm at 82.90°C and 88.05°C, indicating its amorphous nature. On the other hand, the thermogram of poloxamer 188 exhibited an endothermic peak at 54.13°C. The thermogram of Rebamipide nanosuspension showed the endothermic peak of rebamipide at 295.94°C and broad depressed endotherm of chitosan and HPMC.

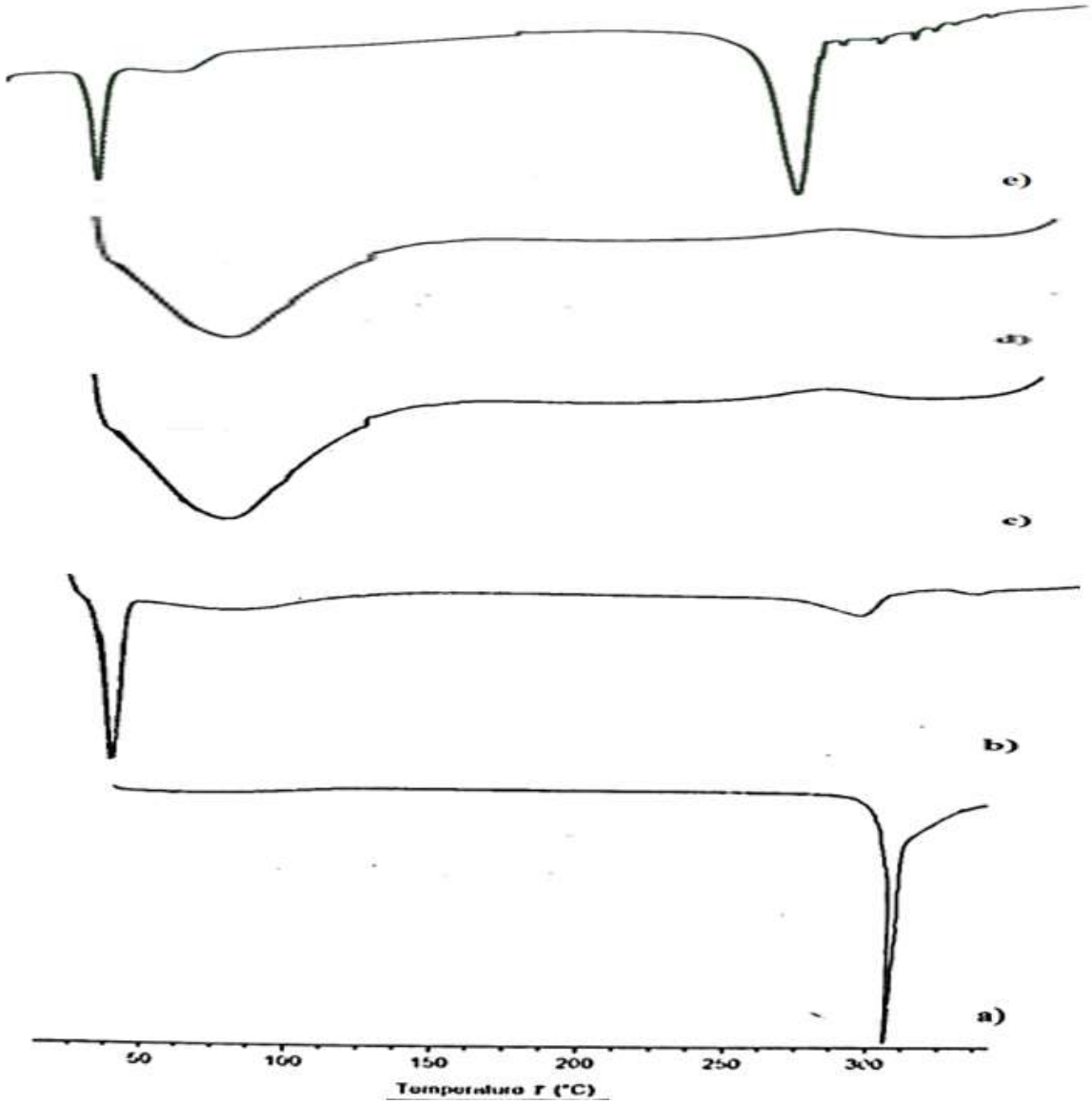

Figure 3: DSC images of a) Rebamipide pure drug, b) Poloxamer 188, c) Chitosan, d) HPMC, and e) Optimized formulation

X-ray powder diffraction (XRD)

The XRD analysis of pure Rebamipide (REB) revealed prominent and well-defined peaks at specific 2θ values, indicating the crystalline structure of REB. In contrast, the XRD patterns of excipients (HPMC, chitosan and poloxamer) exhibited a broad halo pattern, signifying their amorphous nature. Notably, distinct peaks corresponding to Rebamipide were observed in the physical mixture of drug and polymers. According to the XRD results shown in Figure 4, it can be inferred that there were no significant changes in the crystalline structure of REB.

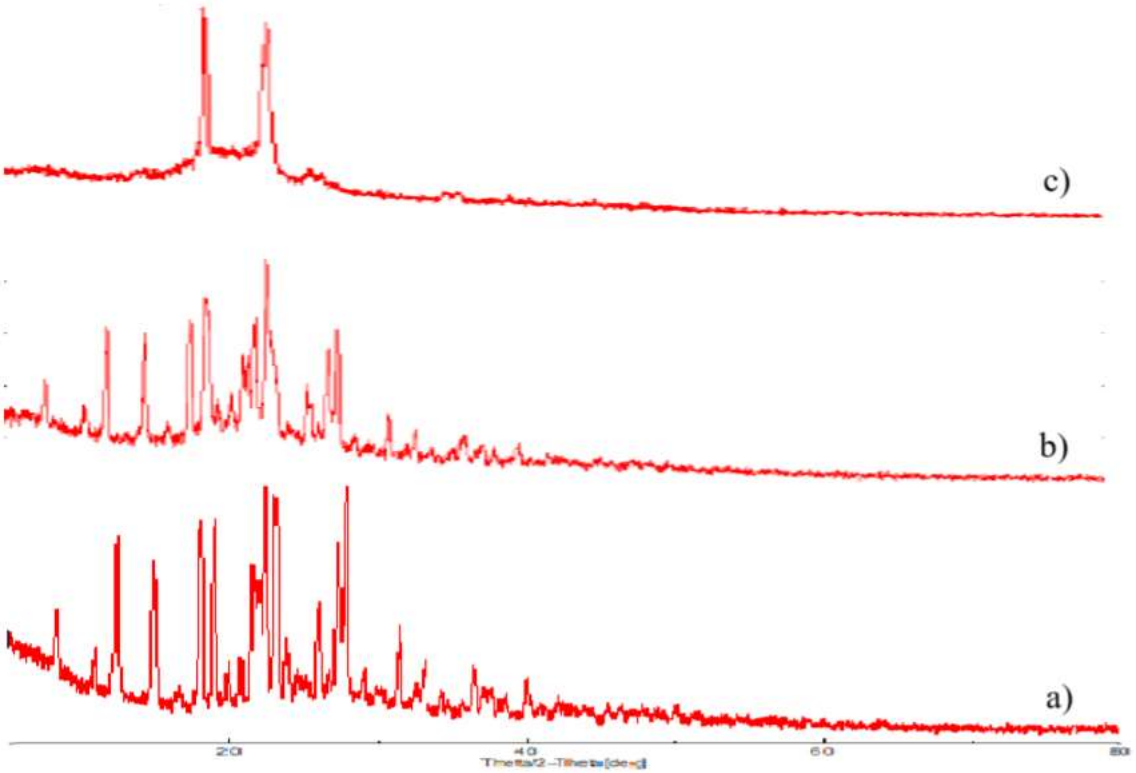

Figure 4: XRD image of a) pure drug REB, b) drug and excipients c) excipients

TEM analysis

TEM (Transmission Electron Microscopy) images of the Rebamipide nanosuspension revealed nanoparticles that were nearly spherical in shape, displaying a smooth surface. This characteristic morphology is illustrated in Figure 5. TEM images provided further evidence that the optimized formulation had nanoparticles with a mean size of not more than 250 nm, emphasizing their small and uniform size.

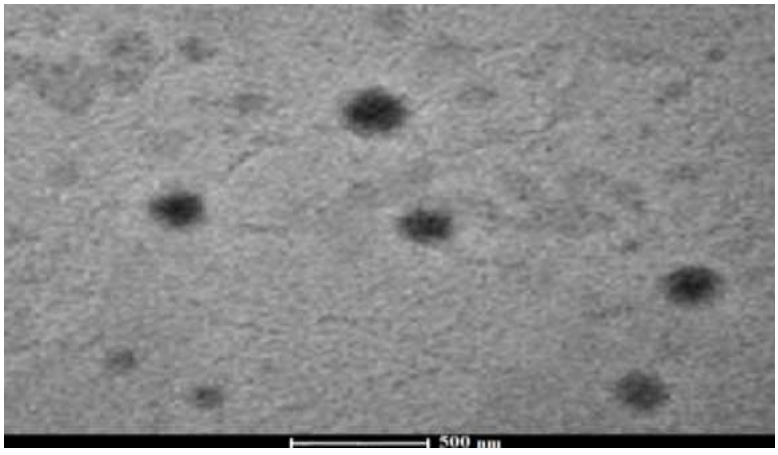

Figure 5: TEM image of the optimized formulation

In-vitro drug release studies

The study compared the release patterns of rebamipide suspension and nanosuspension, showing that the nanosuspension released the drug over a longer period of 24 hours, reaching a total release of 84.7%. Among the mathematical models used to analyze the release behavior, the Peppas-Korsmeyer model was the most accurate in describing how the nanosuspension released the drug. This model suggested that the drug release mechanism follows Fickian diffusion, indicating a controlled and sustained release pattern for the rebamipide nanosuspension.

.

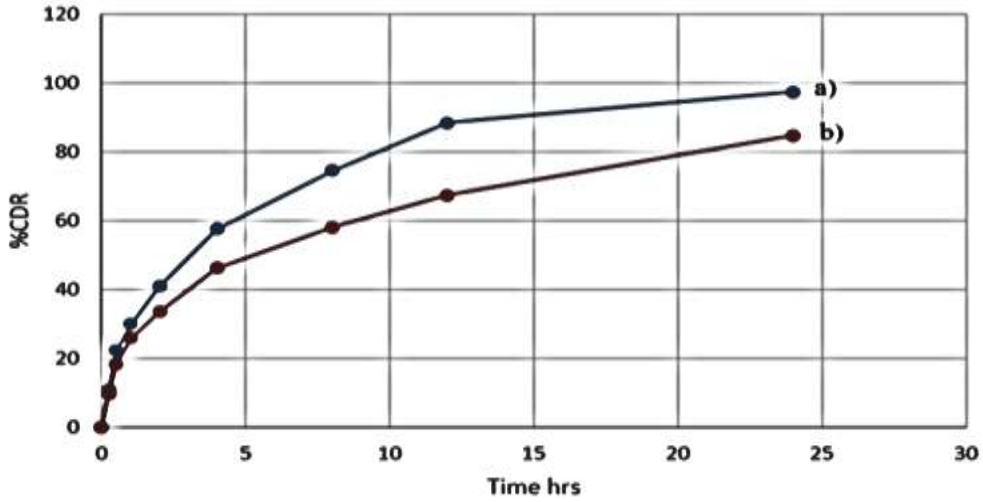

Figure 6: Drug release study of a) rebamipide suspension, and b) optimized formulation

Ex-vivo permeation studies; corneal hydration

The ex vivo drug permeation study on goat corneal membrane demonstrated that the optimized formulation significantly improved drug release, likely due to chitosan's adhesive properties, which enhanced drug adhesion to the corneal surface. Over 8 hours, drug permeation through the cornea reached 86.70%, a notable improvement compared to the conventional suspension. Corneal hydration levels remained within an acceptable range (80.8%), indicating no damage to the corneal layers. This suggests that the optimized formulation enhances drug delivery through the cornea while maintaining corneal integrity.

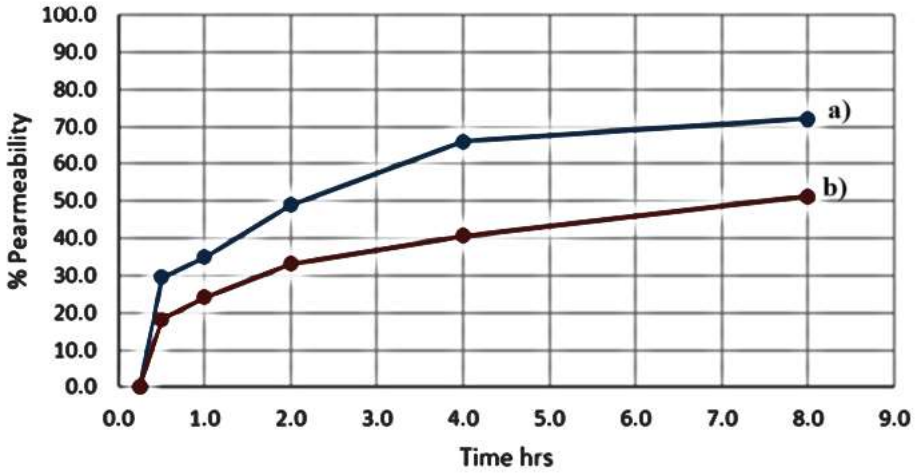

Figure 7: Ex-vivo percentage permeability study of a) REB nanosuspension and b) REB suspension

DISCUSSION

In this study, we successfully developed Rebamipide nanosuspensions using the solvent diffusion method. The optimized formulation exhibited smaller particle size and higher drug entrapment efficiency compared to other formulations. There was a slight reduction in the crystalline nature of Rebamipide in the nanosuspensions due to the presence of excipients. The nanosuspensions had a mean particle size of 196 nm, a low polydispersity index (PDI) of 0.188, and a positive zeta potential of +32.5 mV. These REB nanosuspensions remained physically stable over six months, with entrapment efficiency values ranging from 87.00% to 75.10%. This stability may be attributed to the presence of HPMC and Poloxamer 188, which potentially prevented crystal agglomeration by adsorbing onto the surface of Rebamipide nanosuspension. In vitro drug release profiles indicated that the optimized formulation, consisting 0.6% of water-soluble chitosan, HPMC, and 3% Poloxamer 188, prolonged drug release for up to 24 hours. This extended release may be attributed to the mucoadhesive properties of water-soluble

chitosan. The release data of the optimized REB nanosuspension best fit the Korsmeyer–Peppas model for dissolution in STF buffer at pH 7.4, with a high regression coefficient (R^2 = 0.9899). The release exponent (n) values suggested that drug release followed a Fickian diffusion mechanism. Corneal hydration studies showed that the average hydration level of the cornea for the REB nanosuspension formulation was 80.8%, which remained below 83%, indicating the integrity of the corneas was maintained throughout the experiments. Sterility testing confirmed the absence of microbial growth after 14 days of incubation. Thus, the polymeric system of HPMC and Poloxamer 188 proved effective in developing a stable Rebamipide nanosuspension, primarily through steric stabilization.

ACKNOWLEDGEMENT

We want to thank Diya Labs in Mumbai and Arbro Labs in Delhi for their crucial help in performing TEM, FTIR, and DSC analysis during our study. Additionally, our gratitude goes to the SGT College of Pharmacy, SGT University, Gurugram, for their support in supplying the necessary chemicals and research facilities for our work.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Kesarwani K, Gupta R. Bioavailability enhancers of herbal origin: An overview. Asian journal of tropical biomedicine. 2013; 3(4):253-266.
2. Goyal A, Kumar S, Nagpal M, Singh I, Arora S. Potential of novel drug delivery system for herbal systemfor herbal drugs. Indian journal of pharmaceutical education and research. 2011; 45:225-235.
3. Thakur L, Ghodasra U, Patel N, Dabhi M. Novel approaches for stability improvement in natural medicines. Pharmacognosy Reviews. 2011; 5:48-54.
4. Kinoshita, S., Awamura, S., Oshiden, K., Nakamichi, N., Suzuki, H., Yokoi, N., & Rebamipide Ophthalmic Suspension Phase II Study Group (2012). Rebamipide (OPC-12759) in the treatment of dry eye: a randomized, double-masked, multicenter, placebo-controlled phase II study. Ophthalmology, 119(12), 2471–2478.
5. Kinoshita, S., Oshiden, K., Awamura, S., Suzuki, H., Nakamichi, N., Yokoi, N., & Rebamipide Ophthalmic Suspension Phase 3 Study Group (2013). A randomized, multicenter phase 3 study comparing 2% rebamipide (OPC-12759) with 0.1% sodium hyaluronate in the treatment of dry eye. Ophthalmology, 120(6), 1158–1165.
6. Kase, S.; Shinohara, T.; Kase, M. Effect of topical rebamipide on human conjunctival goblet cells. JAMA Ophthalmol. 2014, 132, 1021–1022.
7. Kase, S.; Shinohara, T.; Kase, M. Histological observation of goblet cells following topical rebamipide treatment of the human ocular surface: A case report. Exp. Ther. Med. 2015, 9, 456–458.
8. Goto, H.; Yamada, M.; Yoshikawa, K.; Iino, M. Ganka- Kaigyoui Notameno Gimon· Nanmon Kaiketusaku; Shindan to Chiryosha Co.: Tokyo, Japan, 2006; pp. 216–217.
9. Nagai, N., Ishii, M., Seiriki, R., Ogata, F., Otake, H., Nakazawa, Y., Okamoto, N., Kanai, K., & Kawasaki, N. (2020). Novel Sustained-Release Drug Delivery System for Dry Eye Therapy by Rebamipide Nanoparticles. Pharmaceutics, 12(2), 155.
10. Kamaleddin MA. 2017. Nano-ophthalmology: Applications and considerations. Nano- medicine. 2017May; 13(4):1459-1472.
11. Ambhore NP, Dandagi PM, Gadad AP. Formulation and comparative evaluation of HPMC and water soluble chitosan-based sparfloxacin nanosuspension for ophthalmic delivery. Drug delivery and translational research. 2016 Feb;6:48-56.
12. Manchanda, S., Sahoo, P. K., & Majumdar, D. K. (2016). Mucoadhesive chitosan-dextran sulfate nanoparticles of acetazolamide for ocular hypertension. Nanotechnology Reviews, 5(5), 445-453.
13. Ahuja, M., Dhake, A. S., Sharma, S. K., & Majumdar, D. K. (2011). Diclofenac-loaded Eudragit S100 nanosuspension for ophthalmic delivery. Journal of microencapsulation, 28(1), 37-45.
14. Shi, Y., Zou, M., An, Y., Ji, Z., Gao, P., & Cheng, G. (2013). A potent preparation method combining neutralization with microfluidization for rebamipide nano-suspensions and its in vivo evaluation. Drug Development and Industrial Pharmacy, 39(7), 996-1004.
15. Guo, Y., Wang, Y., & Xu, L. (2015). Enhanced bioavailability of rebamipide nanocrystal tablets: Formulation and in

vitro/in vivo evaluation. asian journal of pharmaceutical sciences, 10(3), 223-229.
16. Sun, M., Deng, Z., Shi, F., Zhou, Z., Jiang, C., Xu, Z., ... & Xia, S. (2020). Rebamipide-loaded chitosan nanoparticles accelerate prostatic wound healing by inhibiting M1 macrophage-mediated inflammation via the NF-κB signaling pathway. Biomaterials Science, 8(3), 912-925.
17. Patil, O. A., Patil, I. S., Mane, R. U., Randive, D. S., Bhutkar, M. A., & Bhinge, S. D. (2018). Formulation optimization and evaluation of Cefdinir nanosuspension using 23 Factorial design. Marmara Pharm J, 22(4), 587-598.
18. Katara, R., Sachdeva, S., & Majumdar, D. K. (2019). Design, characterization, and evaluation of aceclofenac-loaded Eudragit RS 100 nanoparticulate system for ocular delivery. Pharmaceutical Development and Technology, 24(3), 368-379.\
19. Radhi, Z. A., & Ghareeb, M. M. (2018). Preparation and evaluation of extended release ocular inserts of rebamipide for local effect using casting technique. Iraqi J. Pharm. Sci, 28, 24-36.