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DEVELOPMENT AND CHARACTERIZATION OF DORZOLAMIDE LOADED NOVASOMES FOR THE MANAGEMENT OF GLAUCOMA

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ABSTRACT

A chronic eye condition called glaucoma is characterized by damage to the retinal nerve and progressive vision loss, primarily brought on by high intraocular pressure (IOP). It is one of the main causes of permanent blindness in the world. Currently, the first line of treatment for glaucoma involves pharmacological reduction of IOP through topical administration of drugs. However, because of the anatomical and physiological barriers in the eye, less than 5% of the injected dose usually reaches intraocular tissues. The goal of the research was to develop novasomes to create an efficient medication delivery system for ocular targeting. The solvent evaporation approach was used to develop dorzolamide novasomes utilizing different ratios of cholesterol and free fatty acids (linoleic acid). It was optimized by 3^2 factorial design. Free fatty acid and cholesterol concentrations were the factors under investigation, along with their effects on particle size, polydispersity index, zeta potential, entrapment efficiency, and in vitro drug release. Particle size of all factorial batches was found between 54.1 nm to 434 nm. Batch F6 containing cholesterol and linoleic acid 1:2 proportion showed highest entrapment (98%). The amount of dorzolamide released in vitro from the prepared novasomal dispersions loaded with dorzolamide ranged from 62.76 to 99.76 % and release profile of novasomal batches comparing to eye suspension were better . Ex vivo chicken eye irritation study was performed to check irritation potential of dorzolamide loaded novasomes. In summary, dorzolamide novasomes is an efficient and versatile drug delivery approach which demonstrates significant potential in controlling glaucoma.

INTRODUCTION

Over 70 million individuals worldwide suffer from glaucoma, which is the leading cause of lifelong blindness. Primary open angle glaucoma (POAG) accounts for 74% of cases. With a global frequency of 3.5% in those over 40 and an adjusted odds ratio of 1.73 for every decade of age rise, it is more common in the elderly. The prevalence of glaucoma is expected to rise to nearly 110 million by 2040 because to an aging population and rising life expectancy. (1,2)

Visual loss and progressive optic nerve damage are symptoms of glaucoma. Worldwide, it is the primary cause of irreversible blindness. Treatments for glaucoma aim to slow the progression of the condition by reducing intraocular pressure (IOP), which is often linked to degradation of the glaucoma optic nerve (3,4). Topical medicines, laser treatments, and incisional operations are all options. Early detection, continuing treatment, and treatment adherence are crucial for decreasing disease development and avoiding blindness. Delivering medication to the anterior portion of the eye can be done most easily and minimally invasively by topical administration. Therefore, eye drops are the recommended treatment for a variety of ocular conditions, including as infection, inflammation, glaucoma, dry eye, and allergies, and they make up 90% of the six commercialized products in the global ophthalmic medicine market.(5,6)

Nonetheless, the main restriction of topical administration appears to be its very low effectiveness. Because of the distinctive physiology and anatomy of the eye, drug distribution through the anterior portion is restricted, resulting in diminished bioavailability. Moreover, the physiology, barrier functioning, and design of the cornea all oppose fast drug absorption. To maintain a therapeutic medication level in the tear film or at the site of action, several eye drop applications are required. However, frequent use of extremely concentrated solutions might cause surface cell damage and negative side effects. If the medicine is to have a local effect in the cul-de-sac or raise the proportion of active substance reaching the target area, the duration of its presence in the tear film should be prolonged. Additionally, it is believed that once-daily doses will increase patient compliance.(7,8)

Scientists in the multidisciplinary field of ophthalmology are interested in the challenging problem of drug delivery in ocular therapeutics. Precorneal loss features include tear dynamics,

nonproductive absorption, temporary resident duration in the cul-de-sac, and relative impermeability of the corneal epithelial membrane lead to poor drug bioavailability from ocular dosage forms. Only a limited percentage of the injected medication—perhaps 1% or less of the dosage—gets absorbed by the eyes due to these physiological and anatomical limitations. This forces the physician to recommend high concentrations at frequent intervals, and pulse dosing results in unfavourable outcomes for some ocular formulations and products. Because conventional dosages have the significant drawback of poor bioavailability due to several biological factors that exist to protect the eye and thus limit the entry of ocular drugs, a variety of strategies are being researched, including viscosity enhancement, the use of mucoadhesive polymers, particulate drug delivery, vesicular drug delivery, in situ systems prodrugs, and other controlled systems, like ocuserts.

Novasome is a technique that increases penetration and effectively delivers various chemicals by encapsulating them. (9) Liposomes or niosomes that have been modified by a combination of free fatty acids, cholesterol, and a monoester of polyoxyethylene fatty acids are known as novasomes. (10)

The Novasome (NVS) is a multilayered vesicle that can deliver a significant volume of active component due to its high-capacity central core within a particular size range. Several vaccines produced from novasomes have been granted licenses. (11) They are made up of a mix of cholesterol, free fatty acids, and polyoxyethylene fatty acid monoesters. NVS are multi-bilayer nanovesicles with a great gage cantered core that efficiently load over 80% of hydrophilic or lipophilic materials, reducing the frequency of administration.(10,12) Ophthalmic administration of fenticonazole has been studied as a potential application for NVS recently.(13,14). Because they have an 80% absorption efficiency, they can provide significant amounts of active substances, which lowers the frequency of administration.

As of right now, the sole controllable risk factor for glaucoma is the IOP. By delaying the loss of vision, reducing IOP has been shown to assist in preventing the development and progression of glaucoma. Reducing IOP by 20–50% is the general goal. The goal IOP level decreases as additional risk factors and optic nerve impairment are present. It is important to reevaluate it on a regular basis to determine whether the damage to the optic nerve is stable or increasing(15). By decreasing the production of aqueous humour, dorzolamide (dorzolamide hydrochloride), the first topical carbonic anhydrase (CA) inhibitor to be made accessible for therapeutic use by reducing intraocular pressure (IOP). Bioavailability is low when administered ophthalmic route in the form of eyedrops(16). Various research carried out to overcome the low bioavailability problems associated with topical eyedrops formulation of dorzolamide(17,18)

The goal of this research was to use novasomes to create an efficient medication delivery system for ocular targeting. A cutting-edge and efficient medicine delivery method is novasomes. Novasomes provides activity with a prolonged release. With virtually no negative effects, novasomes improves the treatment's effectiveness and efficiency by improving drug release profile and permeation.

MATERIALS AND METHODS

Dorzolamide was obtained as gift sample from Precise Group, Mumbai. Tween 80, span 80 procured from Loba chemie Pvt Ltd, Mumbai. Dihydrate were purchased from Research- Lab Fine Chem Industries (Mumbai, India) for the preparation of simulated tear fluid. All other reagent used were of analytical grade. Hi-Media Laboratories (Mumbai, India) supplied the dialysis membrane (MW cut off of 12,000–14000).

Formulation of Dorzolamide Loaded Novasomes

The solvent evaporation approach was used to develop dorzolamide novasomes utilizing different ratios of cholesterol and free fatty acids (linoleic acid). Using a water bath at 60 °C, different amounts of drug, FFA, surfactant, and cholesterol were precisely weighed and dissolved in 10 mL of ethanol (Table 2). An ethanolic solution was then progressively mixed with larger amount of phosphate-buffered saline (PBS, pH 7.4) and the mixture was magnetically agitated at the same temperature until all of the ethanol had evaporated. The creation of novasomes was indicated by the abrupt turbidity. To reduce their size, the resulting novasomal dispersions were sonicated for 15 minutes at 25±2 °C.

Optimization of Dorzolamide Loaded Novasomes

The optimization of dorzolamide Loaded Novasomes was performed using 3^2 full factorial design. This investigation evaluated the effects of independent characteristics, such as cholesterol and linoleic acid concentration, on the responses, specifically particle size, PDI, % entrapment efficiency, and Zeta potential of novasomes loaded with dorzolamide. The responses were set at pre-defined goals such as minimum for particle size and poly dispersity index, whereas maximum for % entrapment efficiency and zeta potential. Total of 9 experimental runs varying across two measurable factors and three different concentration levels (Table 1)

Table 1: Formulations Prepared: by 3^2 factorial designs

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Linoleic acid (mg)	100	100	100	200	200	200	300	300	300
Cholesterol (mg)	60	80	100	60	80	100	60	80	100
Span 80: tween 80 (1:1) (mg)	200	200	200	200	200	200	200	200	200
Dorzolamide (mg)	20	20	20	20	20	20	20	20	20

The prepared batches were evaluated for particle size, polydispersity index, zeta potential, drug entrapment efficiency, in vitro drug release, ex vivo permeation, ex vivo drug irritation and in vivo studies

Evaluation and characterization of Dorzolamide loaded novasomes

A uniform, milky white Dorzolamide novasomes was produced and further examined for the following properties.

Particle size, PDI, zeta potential and entrapment efficiency.

The HORIBA Nanopartica SZ100 particle size analyzer was used to assess the particle size analysis of dorzolamide novasomes. Double-distilled water was used to sufficiently dilute the samples in order to attain the required scattering intensity. Particle size is measured using dynamic light scattering (DLS). The dynamic range varies from 0.3 nm to 8 µm based on the sample's physical characteristics.

Polydispersity index

Using the HORIBA Nanopartica SZ100, the polydispersity index was determined at 25^0C and 85% or lower relative humidity (15)

Zeta-potential

Zeta-potential was measured at 25^0C and 85% or less relative humidity using the HORIBA Nanopartica SZ100. The SZ-100 measures the zeta potential of a suspension to quantify the charge on the surface of particles. After injecting the material into a disposable cell, the electrophoretic mobility of the particles is measured to quantify the zeta potential. Zeta potential of the sample is often used to quantify dispersion stability.

Percent entrapment efficiency (EE %)

By indirectly measuring free Dorzolamide (unentrapped Dorzolamide), the percent entrapment efficiency (EE %*) of novasomes loaded with dorzolamide was determined. In short, a cooling centrifuge (REMI) was used to centrifuge 10 mL of the resultant formula for one hour at 4 °C and 21,000 rpm. Both the phases separated and the clear supernatant diluted. Using the calibration curve, the concentration of unentrapped dorzolamide was measured spectrophotometrically at λmax 256 nm. The following formula was used to determine the EE percentage.

$$\text{\% Entrapment Efficiency} = \frac{\text{Total drug - Free drug}}{\text{Total drug}} \times 100$$

Field Emission Scanning Electron Microscopy (FESEM)

Field Emission Scanning Electron Microscopy (FESEM) of optimizes batch was done by FEI Nova NanoSEM 450 at Savitribai Phule Pune University. A scanning electron microscope (SEM) is a kind of electron microscope that creates images by scanning a sample's surface with a concentrated electron beam.

Differential scanning calorimetry (DSC)

The pure dorzolamide, span 80, tween 80, and optimized dorzolamide loaded novasomes were evaluated by DSC to understand the compatibility of drug with surfactants, cholesterol and linoleic acid. A suitable quantity of samples were placed within liquid DSC cells. For the temperature range of 25 to 300 °C, the hermetically sealed samples were heated at a predetermined rate (10 °C/min) while receiving constant heat and nitrogen purging (10 mL/min). A sealed, empty cell was used as a reference, and thermal scans were taken.

In vitro release studies

The produced dorzolamide-loaded novasomal dispersions were subjected to bag dialysis to measure the in vitro release of dorzolamide (typical molecular weight cutoff 14,000Da; Sigma-Aldrich Co.). To put it briefly, the dialysis membrane immersed the whole night submerged in the release medium, which was a pH 7.4 phosphate buffer saline solution with 25% ethanol to maintain the sink condition. Next, a dialysis bag containing 2 milliliters (one milligram of drug) of each formula or dorzolamide suspension was put into amber bottles with a capacity of 25 milliliters for release medium. The bottles were then put in a shaker set to run at 100 rpm and 37±0.5 °C. In an effort to maintain sink condition, 3 mL aliquots were removed at predetermined intervals (0.5, 1, 2, 4, 6, 8h) and replaced with a comparable volume of new release medium. Using a spectrophotometric measurement at λmax 256 nm and the calibration curve, the percent liberated was determined (19).

Chicken eye irritation test

The eye irritation test of optimized 3 batches was performed using freshly excised chicken eyes. F4, F5, F6 batches were selected for the chicken eye irritation test. The saline water was used as standard for comparing with the formulation. HCL was used as the control for the eye irritation test. The 2 hr study was conducted of fresh chicken eyes.

The formulation, saline water and HCL was administered as drops on eyes. After administration of drops the visual inspection was conducted for damage. After 1 hr. of administration dye was used for checking damage incurred to eye. After 2 hr. the visual inspection was carried out to check any damage to eyes. (20)

RESULTS AND DISCUSSION

Particle size of all factorial batches was found below 100 nm. All factorial batches had particle sizes ranging from 54.1 nm to 434 nm. All batches showed particle size between 100 nm except F8 and F9. The particle size of F9 was found to be highest while F4 showed lowest particle size of 54.1 nm. The batches with surfactant and FFA with 1:1 ratio showed excellent size. It was found batch with low concentration of cholesterol and medium concentration of FFA showed lowest size which was F4 batch.

Polydispersity index (PDI)

Polydispersity index was measured by HORIBA Nanopartica SZ100 at 25^0C temp and 85% or less RH. PDI of all factorial batches was found between 0.238 to 365. Lowest PDI was shown by F3 batch 0238 and highest PDI was shown by F9 batch 0.345. It was found that increasing concentration of cholesterol and FFA also increases the PDI.

Z-Average

This is a hydrodynamic parameter and only applicable to particles in dispersion or molecules in solution. Z-Average mean size can be sensitive to even small changes in the sample and will only compare with the size measured by other techniques if your sample is mono-modal, spherical or near-spherical in shape, very narrow and prepared in a suitable dispersant.

Table 2: Factorial batches Z-average

Evaluation parameter	F1	F2	F3	F4	F5	F6	F7	F8	F9
Z- average (nm)	143.2	65.6	81.2	52.5	56.6	61	89.5	166.4	337.4

It was found that as concentration of FFA and cholesterol increased Z-average was also increasing while F9 batch had highest concentration of FFA and cholesterol it showed maximum Z-average. It was found batch with low concentration of cholesterol and medium concentration of FFA showed lowest Z-average which was F4 batch. The p value was found below 0.05 and model was significant.

Zeta-potential

Zeta-potential was measured by HORIBA Nanopartica SZ100 at 25^0C temp and 85% or less RH.

Table 3: Factorial batches Zeta potential

Evaluation parameter	F1	F2	F3	F4	F5	F6	F7	F8	F9
Zeta potential (mV)	-1.3	-1.5	-0.3	-13.4	-8.5	-7.3	-5.1	-3.4	-3.5

It was found that keeping FFA: Surfactant ratio 1:1 gives excellent zeta potential. The zeta of batches F4, F5, F6 was better than others. The p value was found below 0.05 and model was significant.

Percent entrapment efficiency (EE %) of dorzolamide loaded novasomes was calculated by indirect measurement of free Dorzolamide (unentrapped Dorzolamide). %EE of F5 batch was found to be highest while F1 batch was found to be lowest. It was found that increasing concentration of FFA and cholesterol having much effect on %EE.

Scanning electron microscopy-

An electron microscope that employs a concentrated electron beam to scan a sample's surface and produce images is called a scanning electron microscope (SEM). SEM was done at Savitribai Phule Pune University of the optimized batch.

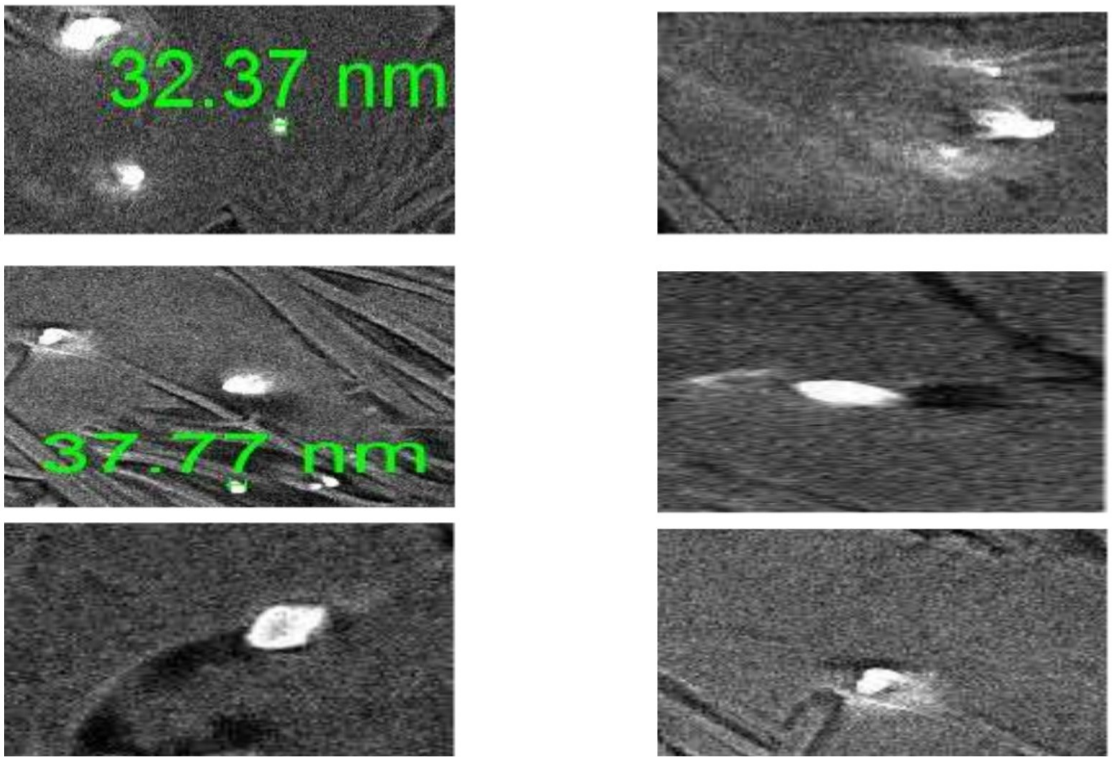

Figure 1: FESEM of dorzolamide loaded novasomes

FESEM of the optimized F4 batch was carried out. The results showed formation of novasomes. The formed novasomes were highlighted and size was measured. Highlighted dots with size show the formation of novasome

In vitro release studies

The produced dorzolamide-loaded novasomal dispersions were subjected to bag dialysis to measure the in vitro release of dorzolamide (typical molecular weight cutoff 14,000Da; Sigma-Aldrich Co.). In vitro drug release of all batches was found between 62.76 to 99.76 %. The drug release was compared with that of drug suspension. The drug release pattern of novasomes showed better release profile than suspension. This was due to small size of novasome than suspension. F6 batch showed best release profile of 99.76 % release followed by F4 batch with 97.9% release.

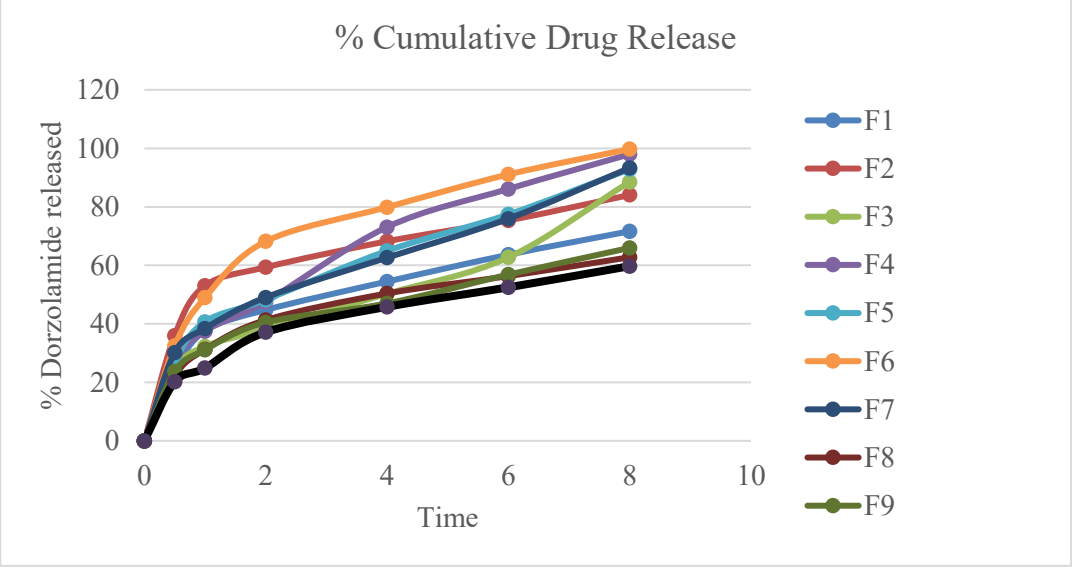

Figure 2: In vitro drug release of dorzolamide loaded novasomes

Chicken eye irritation test

The eye irritation test of optimized 3 batches was performed using freshly excised chicken eyes. F4, F5, F6 batches were selected for the chicken eye irritation test. The saline water was used as standard for comparing with the formulation. HCL was used as the control for the eye irritation test. The 2 hr study was conducted of fresh chicken eyes. After 1 hr. the dye was administered in all eyes to cheek effect of formulation. After administration of the formulation, controll and standard effect was studied. The eye after instilation of HCL was badly damaged while after administration of the formulation and saline water showed no damage.

CONSLUSION

Dorzolamide-loaded novasomes were effectively generated and optimized in this study using a 3^2-factorial design. Dorzolmide loaded novasomes possesses relatively high entrapment efficiency, spherical on shape, high zeta potential values. In vitro

drug release of dorzolamide loaded novasomes showed sustained drug release for 8h. Drug release of dorzolamide loaded novasomes showed higher and sustained drug release when compared with marketed formulation of dorzolamide eyedrops. Ex vivo chicken eye irritation test proves overall safety and corneal tolerability of novasome formulations. From result it was concluded that dorzolamide loaded novasomes could be a promising delivery for the management of glaucoma.

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