Formulation and evaluation of Dorzolamide hydrochloride microsponges loaded in situ gel for ocular administration

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Abstract

Due to the unique structure of the eye, which inhibits the entry of drug molecules into the desired site, the ophthalmic delivery of the drug has been one of the most challenging tasks for a pharmaceutical scientist. For this reason, in this investigation a novel microsponge for an anti-glaucoma drug, Dorzolamide hydrochloride, was formulated as in situ gel for ocular administration with an aim to improve its therapeutic efficacy and reduce the systemic adverse effects. The microsponges were prepared by the quasi emulsion solvent diffusion method and was incorporated into 20% pluronic F-127 which led to consistent in situ gel at 35°C. They were evaluated for physicochemical properties like pH, gelling capacity, gelation time, rheological properties and release profile and ocular irritancy test. The prepared Dorzolamide hydrochloride microsponges showed high entrapment efficiency of 85% and mean particle size of 2 μm with polydispersity index (PDI) of 0.77 which are ideal for ocular delivery. These microsponges is formulated into in situ gel which showed higher therapeutic efficacy compared to free drug in gel. It was found to be non-irritant to the rabbit's eye. These results confirms that Dorzolamide hydrochloride microsponges in situ gels have promising features, benefits and advantages for a novel ophthalmic drug delivery to treat glaucoma.

Keywords: Microsponges; Dorzolamide Hydrochloride; Anti-glaucoma; In situ gel; Pluronic

1. Introduction

The eye is a complex and unique part of the human organs that has been considered as the window to the human soul. Broadly, the human eye is divided into two segments that are anterior and posterior segments[1]. The specific disease conditions of the eye are associated with each of these broad segments. For instance, conjunctivitis, glaucoma, blepharitis, and cataract are some of the diseases that affect the anterior segment of the eye, while diabetic retinopathy and age-related macular degeneration affect the posterior segment [2].

Due to the unique structure of the eye, which inhibits the entry of drug molecules into the desired site, the ophthalmic delivery of the drug has been one of the most challenging tasks for a pharmaceutical scientist. Eye drops accounts for more than 90% of ophthalmic preparations on the markets. They are washed away from the eye and results in low ocular bioavailability (< 5%) after topical administration by different elimination mechanisms [3]. This elimination process includes tear turnover, nasolacrimal drainage, protein binding, systemic absorption, enzymatic degradation and complex penetration barriers (Corneal Barrier, Blood Aqueous Barrier (BAB), and Blood Retinal Barrier (BRB))[4].

One of the main drawbacks in ocular drug delivery is achieving and retaining of optimal concentration of drug at the desired site of action in the eye. Several ophthalmic dosage forms such as ointments, eye drop solutions, gels, and ocular inserts have been investigated in order to prolong the ocular residence time of drugs after the topical application to the eye.
eye. With these formulations, the corneal contact time has been increased to some extent. But, due to blurred vision and poor patient compliance resulted from ointments and inserts, respectively, they have not been fully accepted [5]. Furthermore, drugs that are administered systemically to exert their action in the ophthalmic system also have known to access poorly to the eye tissue [6]. Intravitreal and periocular routes are recommended in order to deliver drugs to the posterior part of the eye. However, there are disadvantages associated with these routes like the frequent intravitreal injections could be painful, thus affecting a patient compliance. The periocular route is easy for administration, but the static and dynamic barriers constitute a problem [7].

Microsponges are polymeric delivery systems of active pharmaceutical agents with potential to be incorporated into a wide variable of pharmaceutical dosage forms, e.g., gels, emulsions, tablets, and capsules, constituted by porous microspheres [8]. Each particle (size range from 5 to 300 μm in diameter) is composed by interconnecting channels forming a non-collapsible structure, with a large porous surface [9,10]. These channels are responsible to maintain the structure of microsponges. These polymeric particles are design to deliver active agents efficiently at the minimum dose and also enhance stability and reduce side effects [11-13].

Ophthalmic in-situ gelling is comprising of environmentally sensitive polymers that will be altered structurally with the small changes in specific conditions like pH, temperature and ionic strength in the environment. In situ forming gels are liquids during instillation into the eye and then undergoes rapid gelation in the cul-de-sac of the eye to form viscoelastic gels in response to environmental changes; lastly release the drug slowly under physiological conditions [14]. Consequently, the residence time of the gel formed in situ will be extended and the drug is released in a sustained manner which leads to enhanced bioavailability, minimized systemic absorption and reduced frequent dosing regimen resulting to improved patient compliance [15,16].

The aim of this study was to formulate novel Dorzolamide hydrochloride loaded microsponges and formulating them into in situ gel for ocular drug delivery, in order to increase the contact time between drug and corneal surface and increase patient compliance.

2. Materials and Methods

2.1. Materials

Dorzolamide Hydrochloride was procured from Century Pharmaceuticals Limited, Gujarat. Ethyl Celluose (EC) (degree of substitution 2.42 to 2.53, viscosity of a 5% w/w solution in 80:20 toluene: ethanol by weight at 25°C, 4cP), Poly vinyl alcohol (PVA) (M.W = 89000), Pluronic F-127 (PF-127), Dialysis tubing cellulose membrane (MWCO=14000) were procured from Sigma-Aldrich Chemicals Pvt. Limited. All other solvents and chemicals used were of analytical standard.

2.2. Methods

2.2.1. Preparation of Dorzolamide hydrochloride loaded microsponges

Microsponge was prepared by the quasi emulsion solvent diffusion method [17]. Firstly, the organic (internal) phase was prepared by dissolving Ethyl Cellulose polymer and Triethylcitrate in 10 ml of Dichloromethane. Triethylcitrate (1% w/v) was used as a plasticizer. Then, the drug was added in the polymeric solution and was ultra-sonicated for 20 min in an ice bath using probe ultrasonicator for homogenous dispersion and particle size reduction of the drug. The polymeric solution was then added drop wise to the aqueous solution previously prepared by dissolving Polyvinyl alcohol (1% w/v) in 100 ml distilled water at 70°C with stirring until it was completely dissolved, then the whole mixture was stirred using overhead stirrer at (2500 RPM) for two hours till complete evaporation of the organic solvent and formation of the microsponges. The mixture was left in a refrigerator for 24 h for complete precipitation of the microsponges, then, the microsponges were filtered, washed with small amount of diluted sodium hydroxide to remove any free drug, washed several times with double distilled water and dried in an oven at 40°C for 48 h, then kept for further studies. The composition is tabulated in Table 1.
Table 1 Formulation of microsponge

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorzolamide hydrochloride (mg)</td>
<td>20</td>
</tr>
<tr>
<td>Ethyl cellulose (mg)</td>
<td>40</td>
</tr>
<tr>
<td>Dichloromethane (ml)</td>
<td>10</td>
</tr>
<tr>
<td>Triethylcitrate (% w/v)</td>
<td>1</td>
</tr>
<tr>
<td>Poly vinyl alcohol</td>
<td>1</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>100</td>
</tr>
</tbody>
</table>

2.3. Characterization of the microsponge formulations

2.3.1. Particle size and size distribution:
A definite dried amount of the Dorzolamide hydrochloride microsponges were suspended in water and were sonicated for one minute to prevent aggregation of the microsponges, then, the mean particle size and size distribution were performed for the microsponges formulation using laser scattering particle size distribution analyser (HORIBA LA-300).

2.3.2. Drug loading (DL%) and entrapment efficiency (EE %)
Accurately weighed 50 mg of the Dorzolamide hydrochloride microsponges were crushed in a mortar, then, transferred to a beaker containing 20 ml of Phosphate buffer pH 7.4 and stirred on a magnetic stirrer for sufficient time to extract and dissolve the drug. Then this solution was filtered using 0.45 μm disc filter and the filtrate was diluted to 100 ml with pH 7.4 phosphate buffer. 1 ml was withdrawn, diluted to 10 ml with pH 7.4 phosphate buffer and was measured spectrophotometrically at 253 nm.

The drug loading (DL %) was calculated using the following equation [18]

\[
\text{Drug loading (\%) } = \frac{\text{Amount of drug in microsponges}}{\text{Amount of microsponges}} \times 100
\]

The entrapment efficiency (EE %) was calculated using the following equation [18]

\[
\text{Entrapment Efficiency (\%) } = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100
\]

2.3.3. Determination of percentage yield
Dorzolamide hydrochloride microsponges were washed, dried and then were weighed accurately. The yield of microsponges was determined by comparing the whole weight of the formed microsponges against the theoretical combined weight of the polymer and drug components.

\[
\text{Percentage yield (\%) } = \frac{\text{Mass of microsponges obtained}}{\text{Total mass of drug and polymer used}} \times 100
\]

2.4. Surface Morphology of Dorzolamide Hydrochloride Microsponges
The morphology and surface characteristics of the Dorzolamide hydrochloride microsponges were studied using the scanning electron microscopy (SEM). One drop of the homogenously suspended microsponges in water was taken and left till complete drying then coated with gold–palladium alloy under vacuum. Coated samples were then examined using SEM.

2.5. In vitro release of drug from Dorzolamide Hydrochloride loaded microsponges
In vitro release test was performed in 50 ml of simulated tear fluid (STF) solution (pH 7.4 at 35±1°C) at 50 rpm using the dialysis method. STF is composed of sodium bicarbonate (0.2%), calcium chloride di-hydrate (0.008%) and sodium chloride (0.67%). A Dialysis tubing cellulose membrane was stretched over the end of a dialysis tube, an accurately
weighed 0.5g of the microsponges were placed on the membrane in the dialysis tube, which was suspended so that the membrane was just below the surface of the buffered dialysis solution, the assembly allowed to shake at 50 rpm at a temperature maintained at 35 ± 1°C. Samples of 2 ml were withdrawn from the release medium at different time intervals (1,2,3,4,5 and 6 h) and were analysed spectrophotometrically at 253 nm. The withdrawn samples were replaced by equal volumes of the STF solution at the same temperature to maintain sink conditions. The experiment was conducted independently in triplicate. Similar studies were carried out for release of pure drug also.

2.6. Development of dorzolamide hydrochloride loaded microsponges in situ gels

Pluronic F127 (PF-127) hydrogel containing Dorzolamide Hydrochloride loaded microsponges equivalent to 2% w/w of the drug were prepared by the cold method [19]. The PF-127 (20% w/v) concentrations of plain gels had the best gelling properties and was used or medicated gel formulation. The weighed amount of PF-127 was slowly added to double distilled water with gentle mixing. The mixture was left in refrigerator at 4°C overnight for complete swelling of the polymer. After the formation of a clear viscous solution, the accurately weighed amount of microsponges was added to the cold solution and were mixed gently with a glass rod. The solution was sonicated for 1 min at 4 °C to form a homogenous gel.

The free drug in gel was also prepared by adding the calculated amount of the drug to a definite volume of double distilled water and then was ultra-sonicated for 2–3 min to decrease the size of drug crystals to about 5–10 μm, after that, the calculated amount of PF-127 powder was added to the preformed suspension, mixed gently and left overnight in refrigerator at 4°C for complete swelling and dissolution of the polymer in water.

2.7. Evaluation of in-situ gels containing dorzolamide hydrochloride loaded microsponges

2.7.1. Determination of the pH

The pH of the formulations were determined in triplicate using calibrated pH meter. The average reading was recorded.

2.7.2. Determination of the gelation time

The gelation time was determined by tube inversion method [20].2 ml of the preparation maintained at 4°C was placed in a test tube, the test tube was placed in water bath maintained at gelation temperature (35°C ± 1), and the in situ gel was observed for gelation by inverting the test tube at time intervals.

2.7.3. Determination of the gelling capacity

The gelling capacity was determined by placing a drop of the in situ gel in a test tube containing 2 ml of freshly prepared simulated tear fluid (pH 7.4) equilibrated at 35 ± 1°C, the time taken for its gelling formation then dissolution of the gel was visually observed and the gelling capacity was evaluated as shown in Table 2.

<table>
<thead>
<tr>
<th>Score</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>No gelation</td>
</tr>
<tr>
<td>+</td>
<td>The gel formed after few minutes and dissolved rapidly</td>
</tr>
<tr>
<td>++</td>
<td>Immediate gelation and remains for few hours</td>
</tr>
<tr>
<td>+++</td>
<td>Immediate stiff gelation which remains for extended period of time</td>
</tr>
</tbody>
</table>

2.7.4. Determination of the rheological behaviour

Viscosity of the prepared gels were determined using a Brookfield Programmable Rheometer. The viscosity was determined at different shear rates from 10 to 50 rpm and then in a descending order (from 50 to 10 rpm) keeping a period of 10 s at each rpm. The samples were equilibrated at 35 ± 1°C prior to each measurement. The viscometer was fitted with T-F spindle 96 and the viscosity was investigated. All measurements were performed in triplicates.
2.7.5. In vitro release

In vitro release test was performed in 50 ml of simulated tear fluid (STF) solution (pH 7.4 at 35 ± 1 °C) at 50 rpm using the dialysis method. STF is composed of sodium bicarbonate (0.2%), calcium chloride di-hydrate (0.008%) and sodium chloride (0.67%) [21]. A standard Dialysis tubing cellulose membrane was stretched over the end of a dialysis tube, an accurately weighed 0.5 g of each of the prepared microsponges gels (each corresponding to 20 mg of the drug) were placed on the membrane in the dialysis tube, which was suspended so that the membrane was just below the surface of the buffered dialysis solution, the assembly allowed to shake at 50 rpm at a temperature maintained at 35 ± 1 °C. Samples of 2 ml were withdrawn from the release medium in the beaker at different time intervals (1, 2, 3, 4, 5 and 6 h) and were analysed spectrophotometrically at 253 nm against a blank similarly treated. The withdrawn samples were replaced by equal volumes of the STF solution at the same temperature to maintain sink conditions. The experiment was conducted independently in triplicate.

2.7.6. Kinetic analysis

Kinetic analysis of the in vitro release data was done in order to determine the drug release mechanism. In vitro release data was fitted to a zero-order (\(m_0 - m = Kt\)), first order (\(\log m = log m_0 - Kt/2.303\)) and Higuchi model (\(m_0 - m = Kt^{1/2}\)) where m is the amount of the drug remaining in the formulation at time t and m0 is the initial amount of the drug in the formulation. The regression coefficient values (r²) were calculated for all the models. Korsmeyer–Peppas equation (\(M_t/M_0 = Kt^n\)) was used to study the diffusion mechanism by analyzing the diffusion exponent “n”. If n ≤ 0.45, the release follows fickian mechanism, if 0.5 ≤ n ≤ 0.8, the release follows non fickian mechanism [22].

2.7.7. In vivo ocular irritancy test in albino rabbits

Albino rabbits were used for this study. The animals were procured from the animal house of Tamil Nadu Veterinary and Animal Sciences University (TANUVAS). The animals were kept under standard living conditions (day/night rhythm) 7.00 a.m to 7.00 p.m, 26 ± 1 °C, in polypropylene cages. The animals had free access to pellet diet. The experimental protocol was approved by the Institutional Animal Ethical Committee. The test was conducted according to the modified Draize test [23]. All the glassware used in the experiment were sterilized by heating and all formulations were prepared under sterile conditions. Three rabbits were used in this experiment. One drop (50 μl) of the microsponges in situ gel formulation was instilled into the lower cul-de-sac of the right eye of each rabbit. The untreated contra-lateral left eye was used as a control. The eyelids were gently held together for about 10 s to avoid the loss of instilled preparations. Each animal was observed for ocular reactions (redness, swelling discharge, conjunctival chemosis, iris and corneal lesions) at 5, 15, 30 min and 1, 2, 3, 6, 9, 12, 24 h post instillation.

The following scores were used to evaluate the irritation [24]. A score of 2 or 3 in any category was considered as an indicator of clinically significant irritation.

- 0: No redness, no inflammation or excessive tearing
- 1: Mild redness with inflammation and slight tearing
- 2: Moderate redness with moderate inflammation and excessive tearing
- 3: Severe redness with severe inflammation and excessive tearing

3. Results and Discussion

3.1. Preparation and characterization of microsponges formulations

Dorzolamide hydrochloride microsponges were prepared by the quasi emulsion solvent diffusion method using Ethyl cellulose (EC) polymer which is an ideal choice as it is biologically inert, non-irritating, non-mutagenic, non-allergenic, non-toxic and non-biodegradable polymer. Quasi emulsion solvent diffusion method appears to be easy, robust and reproducible and features an advantage of eliminating solvent toxicity.

3.2. Particle size analysis and size distribution

The Dorzolamide hydrochloride microsponges formulation was subjected to particle size analysis using particle size analyser which employed scattering light intensity at 74 kcps count rate. The particle size lies between the ranges of 1641 nm – 8510 nm and the PDI value was found to be 0.767. The average particle size was found to be 1780 nm. The particle size distribution graph is shown in Figure 1.
3.3. Zeta potential analysis

Zeta potential analysis was performed to get the information about the surface properties of the microsponges. All microsponges prepared were negatively charged, indicating the presence of ethyl cellulose at the surface of all microsponges formed. Studies have cited that polymers with charged density can serve as good mucoadhesive agents. It has also been reported that anion polymers are more effective bioadhesive than polycations or non-ionic polymers. Zeta potential of the Dorzolamide hydrochloride microsponge formulation was found to be -12.4 mV with negative polarity and shown in Figure 2.

3.4. Percentage production yield

The production yield of the Dorzolamide hydrochloride microsponge formulation was found to be 88%.

3.5. Percentage drug loading efficiency

The obtained drug loading efficiency of the Dorzolamide hydrochloride microsponge formulation was found to be 78%.

3.6. Entrapment efficiency

Entrapment efficiency of the Dorzolamide hydrochloride microsponge formulation was found to be 85%.

3.7. Surface morphology

Scanning electron micrographs of the Dorzolamide hydrochloride microsponges formulation was assessed using High Resolution Quanta 200F Scanning Electron Microscope.
Scanning electron micrographs of optimized Dorzolamide hydrochloride microsponges formulation is shown in Figure 3, which clearly demonstrates that the particles are within the size range of 2 μm and that they are hollow with spherical shape and porous surface. Minor drug particles were also adsorbed on the surface of microsponges.

### 3.8. In vitro diffusion studies

The in vitro release studies of the Dorzolamide hydrochloride microsponges formulation compared to the release of free drug are shown in Figure 4. The free drug has shown to exhibit a significantly higher and faster release than from microsponge formulation. The free drug showed 67.54% of cumulative release after one hour whereas the microspounge
formulation showed 21.75% drug release after one hour. After 2 h, almost all free drug was released about 88.32% from the membrane. Ethyl cellulose polymer was found to retard the release of the drug from microsponges to a large extent. This could be attributed to that the microsponges retarded the drug release due to inclusion of the drug within the voids of the microsponges. These voids acted as a drug reservoir and prolonged the release. The polymer concentration led to increase in the wall thickness and the size of the prepared microsponges, resulting in the reduction of surface area and retardation of the drug release from the microsponges. Also, the slow drug release from microsponges may be attributed to the hydrophobic and floating properties of the microsponges leading to reduction of the drug release.

3.9. Evaluation of the dorzolamide hydrochloride loaded microsponges in situ gel

3.9.1. Determination of the gelling capacity

The formulation (20%w/v PF-127) showed “++” grade of gelling capacity which is a very satisfactory grade. So, this concentration was used for microsponges gel formulations due to its best gelling capacity characters. The results of the gelling capacity of different microsponges gel formulations was observed and it was noticed that medicated in situ gel formulations were gelled immediately when exposed to STF at 35 ± 1°C and it retained for about 4 to 5 h.

3.9.2. Determination of the gelation time

The ideal in situ gelling system is the system which is gelled rapidly on exposure to body temperature to prevent its quick removal by tear fluid. Dorzolamide hydrochloride loaded microsponges in situ gels formulation showed quick gelation time of 23 to 24 s.

3.9.3. Determination of the pH

The ideal pH for an ophthalmic preparation should be in the range of 7.2 ± 0.2. The pH values of the prepared gel formulation was measured and found to be 7.05 ± 0.54 which is in optimal range. However, the limited buffering capacity of the tears is able to adjust the pH values to the physiological pH if it ranged from 3.5 to 8.5. Therefore the prepared Dorzolamide hydrochloride loaded microsponges in situ gel is adequate for ocular application because they were not buffered and could be adjusted to the physiological pH values by tears.

3.9.4. Determination of the rheological behavior of the dorzolamide hydrochloride loaded microsponges in situ gels

The rheological behavior of the Dorzolamide hydrochloride loaded microsponges in situ gels formulations is cited in Figure 5. It was found that the formulations exhibited pseudoplastic flow characteristics (shear thinning systems); the viscosity was increased at low shear rates and decreased under conditions of high shear rates. An advantage of shear thinning formulations is that they have a high viscosity in the open eye, stabilizing the tear film. When blinking occurs, such polymers thin, preventing the feeling of irritation that would occur with high viscosity Newtonian fluid and thus allow a good distribution of the formulation over the surface of the eye.
3.9.5. *In vitro* release of drug from dorzolamide loaded microsponges *in situ* gel formulations

Figure 5 Rheological behaviour of the Dorzolamide hydrochloride loaded microsponges *in situ* gel

Figure 6 *In vitro* release of drug from Dorzolamide Hcl microsponges loaded *in situ* gel formulations compared to the release of Dorzolamide hydrochloride pure drug in gel. Incorporation of the medicated microsponges in pluronic gels enhanced the drug release as PF-127 gel decreased the hydrophobic characteristics of the microsponges. This is because PF-127 is non-ionic polymeric surfactant which led to increase in the wettability of the microsponges.

3.10. Kinetic analysis

Table 3 Kinetic analysis of the *in vitro* release data of Dorzolamide hydrochloride

| Kinetic analysis of the *In vitro* release data of Dorzolamide hydrochloride microsponges loaded *in situ* gel formulations |
|------------------|------------------|-----------------|------------------|------------------|
| Zero-order (r^2) | First-order (r^2) | Higuchi diffusion model (r^2) | Korsmeyar-Peppas (r^2) | Korsmeyar-Peppas diffusion exponent (n) |
| 0.9332           | 0.9957           | 0.9873          | 0.9869           | 0.62             |

In order to obtain the mechanism of drug release, the data was fitted according to different release models and the correlation coefficients (r^2) were calculated and shown in Table 3. The drug release from the microsponges *in situ* gel
followed first order release kinetics model. The diffusion exponent “n” of the Korsmeyer–Peppas equation was $0.5 \leq n \leq 0.8$ which indicated anomalous diffusion or Non-Fickian diffusion mechanism.

### 3.11. In vivo ocular irritancy test on albino rabbits

No signs of ocular irritation such as redness, tearing or swelling were observed in Dorzolamide loaded microsponges in situ gel formulations indicating that it is not irritant and suitable for ocular administration.

### 4. Conclusion

Dorzolamide hydrochloride microsponges were prepared by the quasi emulsion solvent diffusion method with good stability. The microsponges were spherical porous particles as shown by SEM analysis and exhibited a mean particle size of about $2 \mu m$ which is ideal for ocular administration. These stable Dorzolamide hydrochloride microsponges were incorporated into (20% w/v) pluronic F-127 in situ gels. As these prepared gels exhibit pseudoplastic rheology, it is more ideal for administration to the eyes. The in vitro release kinetics of the in situ gel formulations followed first order kinetics model. The formulated Dorzolamide hydrochloride microsponges in situ gel did not cause any irritation to the rabbit eyes even after repeated administration. So, these novel Dorzolamide hydrochloride microsponges in situ gel formulations could be a promising topical ocular drug delivery system for the treatment of glaucoma without causing any systemic adverse effects.

### Compliance with ethical standards

**Disclosure of conflict of interest**

No conflict of interest to be disclosed.

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