ORIGINAL RESEARCH PAPER

Design, Development and Evaluation of Controlled Release Levobunolol Hydrochloride Ocular Inserts for Glaucoma Therapy

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Abstract
The objective of present investigation was to prepare ocular inserts containing levobunolol hydrochloride by solvent casting method using different polymers ratios of ethyl cellulose and Eudragit RL100 to increase the contact time and to achieve controlled release, thereby reducing the frequency of administration, which ultimately improves the therapeutic efficacy. Infrared spectroscopic studies were carried out to rule out the possibility of drug-polymer interaction. The ocular inserts were prepared using moulding technique and the prepared ocular inserts were evaluated for their physical properties, ocular irritation, stability, in vitro and in vivo drug release studies. The in vitro drug diffusion from the inserts was studied using the classical biochemical donor-receptor compartment model, fabricated in the laboratory with commercial semi permeable membrane. All the prepared ocular inserts were uniform in thickness, weight, and drug content. The ocular inserts possessed good tensile strength. All the batches of inserts exhibited zero order drug release pattern. The mechanism of release was found to be diffusion with swelling. The formulation F2, which contained 250 mg ethyl cellulose and 300 mg Eudragit RL100, exhibited optimum physicochemical properties and release profile, and hence was subjected to ocular irritation and in vivo studies in albino rabbits. The optimized formulation F2 showed highest correlation between the in vitro and in vivo drug release ($R^2 = 0.962$).
INTRODUCTION

Glaucoma is characterized by slow progressive degeneration of the retinal ganglion cells and the optic nerve axons, leading to progressive, irreversible loss of vision. Glaucoma is a disease in which the optic nerve is damaged; it is often, but not always, associated with increased pressure (IOP > 21 mmHg) of the fluid in the eye. The intraocular pressure (IOP) is usually elevated and, if left untreated, can result in further optic nerve damage.1

Glaucoma is known as 'silent thief of sight', as it is associated with loss of vision. Glaucoma normally occurs gradually over a long period of time and is often only predictable when the disease is quite advanced. Once lost, the damaged visual field cannot be recovered. It is the second leading cause of blindness in the world. Glaucoma affects 1 in 200 people aged fifty and younger, and 1 in 10 over the age of eighty. If the condition is detected in early stage, it is possible to control the development or slow down the progression with medical and surgical means. WHO estimated the global population with high IOP (>21 mm Hg) as 104.65 million and the number with chronic open angle glaucoma at 13.5 million.2

Glaucoma therapy includes lowering of IOP to normal level, either by reducing secretion of aqueous humor or by promoting its drainage. Topical beta-blockers are the frontline drugs used in the treatment of glaucoma. These drugs block beta-2 receptors located on ciliary epithelium, thereby decreasing the aqueous humor formation without affecting pupil size, tone of ciliary muscle, or outflow facility. This effect is probably due to down regulation of adenylycyclase due to beta 2 receptor blockade and a secondary effect due to reduction in ocular blood flow.3

Delivery of drug to the eye has remained as one of the most strenuous task for pharmaceutical scientists. The intraocular bioavailability of the drug through conventional eye drops is very poor due to factors such as nasolachrymal drainage, lacrimation, drug dilution with tear fluid, tear turnover and conjunctival absorption. Binding of drugs to protein also contributes to loss of drugs through the precorneal parallel elimination loss pathway. Consequently, only a small amount of (1-3%) actually penetrates the cornea and reaches the intraocular tissue.1

Use of controlled release drug delivery system can improve the corneal residence time of the drug. One of the methods to increase the precorneal residence time of the drug is by dissolving or dispersing the drug in the polymeric matrices. These matrix delivery systems offer higher resistance to drug loss through drainage and tear flow compared to conventional ophthalmic formulations. They also produce reliable drug release in the cul-de-sac. These systems also reduce systemic drug absorption due to sustained release of the drug.4 Ocular inserts are defined as sterile preparations, with solid or semisolid consistency, that are placed into cul-de-sac. Their size and shape are specially designed for ophthalmic application. They are usually made up of polymeric vehicle containing drug and are mainly used for topical therapy.5

Levocabunolol hydrochloride is a nonselective beta-adrenoceptor antagonist used in the treatment of chronic open-angle glaucoma.6,8 It reduces both elevated and normal intraocular pressure in patients with or without glaucoma. In patients with elevated intraocular pressure, levobunolol reduces it by approximately 25-40% from the baseline. As the drug is a nonselective beta-blocker, it produces both systemic pulmonary and cardiovascular effects, such as bronchoconstriction, decrease in blood pressure and heart rate, following topical application to the eye.7,8 The mechanism of reduction of intraocular pressure is believed to be due to reduction of the production of aqueous humor via blockage of endogenous catecholamine-stimulated increases in cyclic adenosine monophosphate (AMP) concentrations within the ciliary processes.6,8

In the present study, an attempt was made to develop and evaluate the ocular inserts of levobunolol hydrochloride with release profiles optimized to reduce frequency of dosing and therefore, overcome the side effects caused due to repeated administration of the drug, which ultimately improves the patient compliance.
MATERIALS AND METHODS
Levobunolol HCl was procured as a gift sample from Piramal health care Pvt. Ltd, Mumbai (India). Ethylcellulose and Eudragit RL 100 were obtained from SD Fine Chem Ltd, Mumbai (India) and Evonik Degussa India Pvt. Ltd, Mumbai (India), respectively. All other chemicals and solvents used were of analytical grade.

Drug-polymer Compatibility
The compatibility between levobunolol HCl and the selected polymers was determined by FTIR peak-matching method using a FTIR spectrophotometer (Shimadzu, Japan), following potassium bromide disc method.9

Preparation of Ocular Inserts
Ocular inserts containing Levobunolol HCl were prepared by casting method using a glass mould10 with varying ratios of selected polymers as shown in the Table 1.

Table 1 Formulation design of ocular inserts using Ethyl cellulose and Eudragit RL100

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity in each batch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levobunolol HCl (mg)</td>
<td>60  60  60  60  60</td>
</tr>
<tr>
<td>Ethyl cellulose (mg)</td>
<td>150 250 200 150 250</td>
</tr>
<tr>
<td>Eudragit RL 100 (mg)</td>
<td>300 300 350 400 400</td>
</tr>
<tr>
<td>Dibutyl phthalate (mg)</td>
<td>160 160 160 160 160</td>
</tr>
<tr>
<td>Ethanol (mL)</td>
<td>6  6  6  6  6</td>
</tr>
</tbody>
</table>

Ethyl cellulose and Eudragit RL 100 (total weight = 450 to 650 mg) were weighed in requisite ratios and dissolved in ethanol (4 mL) to form a clear solution. Levobunolol hydrochloride (60 mg) was dissolved in 2 mL of ethanol under mild agitation. The ethanolic solution of drug was added to polymeric mixture with gentle agitation. The plasticizer dibutyl phthalate (DBP) was added to the above solution under mild agitation. This mixture was poured into a glass mould and dried at room temperature for 72 h. During drying, the glass mould was covered with inverted funnel plugged with cotton in the stem to ensure slow evaporation of the solvent. After complete drying, stable ethyl cellulose / Eudragit RL (EC/RL) films were formed. Dummy films were also prepared following the same procedure. Films with any imperfections such as entrapped air, differing in thickness, weight or content uniformity were excluded from further studies.

The dried films were cut to circular discs (ocular inserts) of diameter 0.8 cm (area 0.6364 cm²) containing 2 mg of levobunolol HCl, wrapped in aluminium foil, placed in self-sealable polythene bags and stored in the desiccator until further studies.

Physicochemical Evaluation of Ocular Inserts

Thickness and Weight Uniformity of Ocular Inserts
Thickness of 10 final circular ocular inserts was measured using digital screw gauge (Mitutoyo, Japan) and the average as well as standard deviations (SD) were calculated.11 For uniformity of weight, 10 inserts from each batch were selected randomly and weighed individually on electronic balance (Shimadzu Corporation, Japan). The average weight and standard deviation were calculated.11

Folding Endurance
Evaluation of folding endurance is the study to see the folding capacity of the film when subjected to frequent extreme conditions of folding.12 This study was carried out manually by folding and unfolding...
the dried film completely in the middle several times until the film was broken. Folding endurance is expressed as the number of folds required to break or to develop visible cracks on the film. Five films from each batch were used for this study.

**Surface pH**
Surface pH of the films was determined by pH indicator paper after allowing the inserts to swell on 2% agar for 5 h in petriplate. The surface pH was measured by means of a pH paper placed on the surface of swollen film.\(^\text{13}\)

**Swelling Studies**
Ocular inserts (n=3) from each batch were weighed and placed separately in test tubes containing 4 mL of simulated tear fluid (STF, pH 7.4). At regular intervals of time (every 10 min), the films were removed and the excess water on their surface was removed using a blotting paper and again weighed. The procedure was continued till there was no increase in the weight. The swelling index was calculated by dividing the increase in weight by the initial weight and was expressed as percentage.\(^\text{14}\)

**Moisture Absorption**
Three ocular inserts were kept in a desiccator containing calcium chloride at room temperature for 24 h and weighed accurately. Then, they were exposed to 75\% relative humidity by placing them over sulfuric acid (14\%) for a period of 24 h in a desiccator. These inserts were weighed repeatedly until they showed a constant weight.\(^\text{15}\) Percent moisture uptake was calculated using the formula:

\[
\text{Moisture uptake (\%)} = \frac{\text{(Final weight} - \text{Initial weight)}}{\text{Initial weight}} \times 100
\]

**Tensile Strength**
Tensile strength of the ocular inserts was determined with Hounsfeld universal testing machine (Industrial Engineering Instruments, Bangalore, India). The sensitivity of the machine is 1 mg to 500 kg. The test insert of specific size (4 \times 1 cm\(^2\)) was fixed between the two load cell grips. Force was gradually applied at a speed of 100 mm/min (ISI Standard speed) till the film was broken. The tensile strength of the films was taken directly from the dial reading in kilograms. The tensile strength per cm\(^2\) was calculated using the formula:

\[
\text{Tensile strength (kg/cm}\,^2) = \frac{\text{Break force (kg)}}{\text{Cross-sectional area of the sample (cm}\,^2)}
\]

**Drug Content Uniformity**
Six ocular inserts from each batch were dissolved in series of 100 mL volumetric flasks containing ethanol and were filtered. Then, the absorbance was measured at 222.4 nm in a UV spectrophotometer (UV 1700, Shimadzu, Kyoto, Japan) after suitable dilution with STF of pH 7.4 against a blank prepared using a drug-free insert.\(^\text{16,17}\)

**In vitro Drug Release**
The *in vitro* release of drug from the inserts was studied using the classical biochemical donor - receptor compartment model comprising a cylindrical tube (15 mm internal diameter and 100 mm height) and a glass beaker fabricated in the laboratory.\(^\text{16,17}\) The dialysis membrane No.50 (HiMedia Laboratories, Mumbai), soaked overnight in simulated tear fluid (STF, pH 7.4) was tied to one end of open cylinder. This worked as a donor compartment. The insert was placed inside this compartment. The entire surface was in contact with the receptor compartment containing 30 mL of STF in 100 mL beaker. The content of receptor compartment was stirred continuously at low speed maintaining a temperature of 37 ± 1 °C. At specific time intervals, 1 mL of sample was withdrawn from the receptor compartment and replaced with fresh STF (pH 7.4). The samples were analyzed using UV spectrophotometer at 222.4 nm against STF (pH 7.4) as blank.
To analyze the drug release kinetics and mechanism of drug release from the prepared ocular inserts, the in vitro release data of the ideal batch of the ocular insert was fitted into Zero order, First order, Higuchi’s and Peppas’ models. The regression coefficient (R²) values were calculated to select the best-fit model.¹⁸

Sterilization and Sterility Testing
Sterilization of ocular inserts was carried out by placing inserts under ultra violet light in ultra violet germicidal chamber, exposing both sides for 15 min at a 10 cm height from UV lamp.¹⁵,¹⁹ Sterility testing of the ocular films was carried out by aseptic transfer of sterilized films into two different sterile broth tubes. One un inoculated broth tube was taken as negative control (to test sterility of the medium). A laboratory isolated confirmed Staphylococcus aureus strain inoculated in another broth tube, served as positive control (indicative of ability of the medium to support growth). The tubes were incubated for 48 h and observations were made for microbial growth. The results were interpreted with reference to positive and negative controls.

Ocular Irritation
Ocular irritation study was conducted in albino rabbits after prior permission from the institutional animal ethics committee (IAEC approval no. BEA. BPh/05/2007-2008).²⁰-²² Six albino rabbits weighing about 1.5 to 2 kg were used in the study and were examined thoroughly for any pre-existing ocular damage. The sterile optimized batch of ocular insert was then placed in one eye of each animal by gently pulling the lower eyelid away from the eye ball (conjunctival cul-de-sac). The eyelids were then gently held together for few seconds and the animal was released. The other eye, remaining untreated was served as the control. The eyes of each rabbits were examined after 1, 4, 12, 24, 48, and 72 h for irritation, inflammation etc.

In vivo Drug Release
The optimized F2 batch of ocular inserts were placed into the conjunctival cul-de-sac of left eye of six healthy rabbits. The other eye served as the control. At specific time intervals, inserts were carefully removed and analyzed for the residual drug content. The drug remained in the insert was subtracted from initial drug content of the insert to calculate the amount of drug released into the rabbit’s eye. The experiment was conducted in triplicate with a wash out period of one week between the experiments.²³

RESULTS AND DISCUSSION
The aim of the present study was to develop ocular inserts of levobunolol HCl using ethyl cellulose and Eudragit RL 100 to control the drug release for a period of at least 3 days. Fig 1 shows the FTIR spectra of pure drug, and its combinations with different polymers for determination of drug-polymer compatibility. The FTIR spectrum of pure levobunolol HCl (Fig 1a) showed the peaks at 3371 cm⁻¹ (N-H stretch), 2865 cm⁻¹ (C-H stretch), 1184 cm⁻¹ (C=N stretch), 1037 cm⁻¹ (C-O stretch) and 1677 cm⁻¹ (C=O stretch). These peaks were considered as principal characteristic peaks of the drug. All the characteristic peaks of the pure drug were present in different drug-polymer combinations (Fig 1b, 1c and 1d), indicating that there was no interaction between levobunolol HCl and the selected polymers.

The controlled release ocular inserts were prepared and evaluated for various physicochemical properties, drug content, release behavior and in vivo ocular irritation and in vivo release profiles. Various physicochemical properties of the developed ocular inserts are given in Table 2. All the batches of ocular inserts exhibited uniform thickness with minimum standard deviation. The drug loaded ocular inserts of 8 mm diameter exhibited uniform weight. The developed ocular inserts had good folding endurance, and did not show any cracks even after folding for more than 200 times. Higher folding endurance indicates good flexibility of the inserts. Surface pH of all the batches of ocular inserts was within the range of 6.2-7.5, which indicated that the developed inserts may not cause ocular irritation.

In the swelling study, it was found that all the batches of the developed ocular inserts exhibited minimum swelling (Fig 2). This indicates that the developed inserts might not cause any discomfort in the eye as extraneous bodies. The swelling was found to increase with concentration of Eudragit RL 100. This could be attributed to the high water permeability of Eudragit RL100, which is due to the presence of quaternary ammonium groups in its structure.²⁴,²⁵
Fig 1 FTIR spectra of levobunolol HCl (a), combinations of levobunolol HCl and Eudragit RL 100 (b), levobunolol HCl and ethyl cellulose (c), levobunolol HCl, ethyl cellulose and Eudragit RL 100 (d)

Fig 2 Swelling studies of developed ocular inserts
Table 2 Physicochemical parameters of ocular inserts

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Thickness (mm)</th>
<th>Average weight (mg)</th>
<th>Amount of drug present (mg)</th>
<th>Moisture absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.196 ± 0.005</td>
<td>14.24 ± 0.117</td>
<td>1.818 ± 0.017</td>
<td>3.15 ± 1.082</td>
</tr>
<tr>
<td>F2</td>
<td>0.213 ± 0.002</td>
<td>13.44 ± 0.210</td>
<td>1.857 ± 0.010</td>
<td>2.84 ± 0.946</td>
</tr>
<tr>
<td>F3</td>
<td>0.236 ± 0.003</td>
<td>14.14 ± 0.141</td>
<td>1.791 ± 0.010</td>
<td>3.28 ± 0.792</td>
</tr>
<tr>
<td>F4</td>
<td>0.253 ± 0.003</td>
<td>10.16 ± 0.145</td>
<td>1.829 ± 0.025</td>
<td>3.19 ± 0.649</td>
</tr>
<tr>
<td>F5</td>
<td>0.261 ± 0.010</td>
<td>15.13 ± 0.110</td>
<td>1.818 ± 0.017</td>
<td>2.79 ± 0.758</td>
</tr>
</tbody>
</table>

The values are average ± SD (n=3)

The percentage moisture absorbed by the ocular inserts was found to increase with increase in the concentration of Eudragit RL100, which can again be attributed to the high water permeability of Eudragit.\(^\text{24,25}\) The tensile strength values of drug loaded and blank ocular inserts are given in Fig 3. The drug loaded ocular inserts exhibited higher tensile strength values than the blank films. This might be due to the strengthening of polymer chains by the dissolved drug. The tensile strength was found to increase with increase in polymer solubility. The drug content of ocular inserts of diameter 0.8 cm (area 0.6364 cm\(^2\)) was found to be more than 87%, indicating a good uniformity (Table 2).

Fig 3 Tensile strength of drug-loaded and blank ocular inserts

The in vitro release data of levobunolol HCl from different batches of ocular inserts is shown in Fig 4. The release of levobunolol HCl was found to be controlled from all the formulations. But, the release was found to vary with concentration of ethyl cellulose and Eudragit in the inserts. The inserts containing higher concentration of ethyl cellulose exhibited lower drug release, which might be attributed to the hydrophobic nature of ethyl cellulose, which reduces the solvent penetration, leading to reduced diffusion of drug from the matrix. Increase in the concentration of Eudragit RL100 increased the drug release, which might be attributed to the high water permeability of Eudragit RL 100. When these inserts are exposed to the dissolution medium, the solvent penetrates into the free spaces between macromolecular chains. After solvation of the polymer chains, the dimensions of the polymer molecule increase due to polymer relaxation by the stress of the penetrated solvent.\(^\text{26}\) Based on the physical properties, drug content and in vitro drug release profile, the batch F2 containing 250 mg ethyl cellulose and 300 mg Eudragit RL100 was considered as ideal and was used for further studies.
To determine the kinetics of drug release from the developed ocular inserts, the *in vitro* release data of the F2 was fitted into zero order and first order models (Fig 5). It can be seen that the regression coefficient ($R^2$) value was poor for the first order model (0.772). In case of zero order model, the $R^2$ value was near to 1 (0.986), indicating a perfect fit. Hence, it was considered that the release of drug from the developed ocular inserts was following zero order kinetics. The slope value of the zero order plot was found to be 1.303, which gives the release rate constant.

To find out whether the drug release is through diffusion, the *in vitro* release data was plotted according to the classic Higuchi’s equation. The Higuchi’s plot for the batch F2 was found to be linear ($R^2 = 0.980$), indicating a diffusion controlled release (Fig 6a).

To find out the exact mechanism of release, the *in vitro* release data was fitted into Peppas’ model. According to the logarithmic form of Peppas’s equation, the rate of drug release can be expressed as:

$$\log Q = \log K + n \log t$$

Where, $Q$ is the amount of drug released, $t$ is the time, and $n$ is the slope of the linear plot. If the value of $n$ is less than or equal to 0.5, the mechanism of drug release is diffusion without swelling. If the value is
greater than 0.5 and less than 1, the release is through diffusion with swelling, and if it is above 1, the release mechanism is anomalous diffusion, not confirming any of Fick’s laws (non-Fickian).

The Peppas’ plot for the release from ocular insert of batch F2 is shown in Fig 6b. The plot was found to be linear with $R^2$ value of 0.989. The slope value of the plot was 0.709, indicating that the drug release mechanism is Fickian diffusion with swelling.

Sterilization and sterility test of optimized formulations F2 were sterilized by UV radiation and sterility testing was carried out under aseptic conditions. The growth of *Staphylococcus aureus* in positive control indicated that the medium was suitable for microbiological growth. In the negative control tube, there was no growth of microorganisms, which confirmed the sterile condition of the apparatus and glassware used, and also the maintenance of proper aseptic environmental conditions during the test. There was no growth of microorganisms in the samples under test confirming the sterility of the apparatus and glassware used, and also the maintenance of proper aseptic environmental conditions during the test. There was no growth of microorganisms in the samples under test confirming the sterility of ocular inserts. Therefore, the sterilized inserts were considered suitable for *in vivo* studies.

Ocular irritation study was carried out in albino rabbits using the optimized formulation F2, to determine whether the developed inserts cause any irritation and pain after administration. The eyes of the rabbits were inspected visually at specific time intervals after administration of the ocular inserts. There were no signs of redness and continuous blinking of the eyes. No ocular damage or abnormal signs of cornea, iris, and conjunctiva were visible. The ocular irritation scoring for the developed formulation was found to be zero (data not shown). Hence, it was concluded that the developed ocular inserts were non-irritant to the eye.

The *in vivo* drug release study of the optimized formulation batch F2 was carried in rabbits by measuring amount of drug remained in the inserts at periodic time intervals. The drug release from the developed ocular insert after 60 h was found to be 98.23% (Fig 7). An attempt was made to correlate *in vitro* and *in vivo* drug release profiles of batch F2 (Fig 8). From the figure, it can be clearly seen that there is good correlation between *in vitro* and *in vivo* diffusion profiles of the drug from the developed ocular inserts ($R^2 = 0.962$). This proves that the ocular inserts have reproducible drug release in biological environment that matches with *in vitro* drug release. Hence, it can be concluded that the *in vivo* drug release behaviour of the ocular inserts can be predicted by observing the *in vitro* release pattern. This also indicates a reproducible therapeutic response.

**CONCLUSIONS**

Based on the results, it can be concluded that effective treatment of glaucoma is possible by formulating ocular inserts of levobunolol hydrochloride that release the drug in a controlled manner over a prolonged period of time. The developed ocular inserts of levobunolol hydrochloride are safe for ocular use and have ability to prolong the drug release, which may, in turn, reduce the dosing frequency, improve the therapeutic efficacy and may enhance the patient compliance. To prove the therapeutic efficacy the
developed ocular inserts, *in vivo* pharmacokinetic and pharmacodynamics studies in animal models and also in human volunteers are required.

**Fig 7** *In vivo* drug release from the ideal batch of ocular inserts (F2) in rabbits

**Fig 8** Correlation between *in vitro* and *in vivo* drug release profiles of ideal batch of ocular inserts (F2)

**DECLARATION OF INTEREST**
It is hereby declared that this paper does not have any conflict of interest.

**REFERENCES**
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