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# Characterization of Best Optimized Levobunolol Hydrochloride Occusert Formulations with Special Reference to Glaucoma Treatment

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## ABSTRACT

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The aim of present study was to characterize best optimized Levobunolol HCl occusert formulations with special reference to glaucoma treatment, biologically. Levobunolol HCl occuserts were prepared using hydroxypropyl methylcellulose (HPMC, 3% & 4%), poly vinyl pyrrolidone (PVP, 1%), methyl cellulose (MC, 1% & 2%) as polymers by solvent casting technique with objectives of increasing contact time, achieving controlled release, reduction in frequency of administration and greater therapeutic efficacy. Based on their physiochemical and *in-vitro* drug release characterization, formulations ($F_2$ & $F_1$) containing 4 % and 3 % HPMC were selected as best optimized formulations. Further, these formulations were subjected to biological characterization such as microbial sterility testing, Modified Draize eye irritation and *in-vivo* drug release studies for their efficacy, reliability and clinical safety. Finally, accelerated stability testing (aging study) was also carried out. Results of these studies revealed that, occusert formulations ($F_2$ & $F_1$) passed the test for sterility, there was no irritation to the sensitive ocular tissues and *in-vivo* study had released drug contents, 77.14 % and 69.83 % ($F_2$ & $F_1$) respectively over an extended period of 12 hr. Finally, the ocular inserts were found stable and there was no effect on the drug content for a period of 2 months. It was found that, best optimized polymeric occuserts have appreciable strength biologically.

**Keywords:** Characterization, Glaucoma, Levobunolol HCl, Occusert.

## INTRODUCTION

Glaucoma is a group of diseases of eye characterized by damage to the ganglion cells and optic nerves. Glaucoma is usually described as open angle and/or closed angle (angle closure). These terms are based upon the mechanism of obstruction of outflow of aqueous humor and help clinicians for developing treatment strategies. A third type is congenital glaucoma, which results from developmental ocular abnormalities and occurs in less than 2% of patients[1]. Among asians, the incidence of closed angle glaucoma is almost twice as high as that for caucasians. The true picture of disease incidence in asian continent remains to be elucidated as yet. Current studies indicate the involvement of excitatory and inhibitory neurotransmitters *viz.* glutamate, gamma amino butyric acid (GABA) and glycine in the development of glaucoma. The excess supply of excitatory neurotransmitter glutamate is particularly linked to glaucoma. Apoptosis or genetically programmed cell death has also been implicated as a mechanism for progression of glaucoma[2].

Levobunolol HCl is a non-cardioselective β-adrenoceptor blocking agent, equipotent at both $\beta_1$ and $\beta_2$ receptors. Levobunolol HCl does not have significant local anesthetic (membrane-stabilizing) or intrinsic sympathomimetic activity. β-adrenergic receptor blockade reduces cardiac output in both healthy subjects and patients with heart disease. The primary mechanism of ocular hypotensive action of levobunolol HCl in reducing intra-ocular pressure (IOP) is most likely a decrease in aqueous humor production. Levobunolol HCl ophthalmic solution has been shown to be effective in IOP and may be used in patients with chronic open- angle glaucoma or ocular hypertension. Its side effects are transient burning sensation, blurred vision, mild ocular irritation, bronchospasm, reduction in resting heart rate and blood pressure[3]. Previous literature on Levobunolol HCl reported that, only *in-situ* gels were prepared by *pH* induced gelling system for glaucoma treatment[4].

However, based on their design, physiochemical and *in-vitro*

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drug release characterization, Levobunolol HCl occusert formulations ($F_2$ & $F_1$) containing 4 % and 3 % HPMC were selected as best optimized formulations. Further, these formulations were subjected to biological characterization such as microbial sterility testing, Modified Draize eye irritation and *in-vivo* drug release studies for their efficacy, reliability and clinical safety. Finally, accelerated stability testing (aging study) was also carried out.

## MATERIAL AND METHODS

### Chemicals:

Levobunolol HCl was procured from BAL Pharma Ltd., Bangalore, Hydroxy Propyl Methyl Cellulose (HPMC 15cps) from Colorcon Ltd., Goa and Methyl Cellulose (MC), Ethyl Cellulose (EC), Poly Vinyl Pyrrolidone (PVP K32) and Dibutyl Phthalate (DBP) from S. D. Fine Chemicals, Bombay. Solvent used was distilled water, which is permissible for eye delivery. All other reagents and solvent used for study were of analytical grade.

### Instruments:

U.V. Visible Spectrophotometer-1700 (Shimadzu, Japan), Digital Balance (Shimadzu, Japan), Magnetic Stirrer, Overhead Stirrer and Cyclone Mixer (Remi, Mumbai), Hot Air Oven (Tempo Pvt. Ltd., Mumbai) instruments were used for this study.

### Preparation of Levobunolol Hcl Polymeric Occuserts:

### Selection of best polymer composite for occuserts:

Polymers were selected on the basis of their solubility or dispersibility, compatibility of polymer and Levobunolol HCl with solvents. Solvent evaporatability was also taken into consideration.

### Preparation of blank polymeric occuserts:

The various formulations of blank polymeric occuserts were prepared using various concentrations of HPMC, MC and PVP both alone and in combination as shown in **Table-1**, by solvent casting technique[5,6].

### Incorporation of drug:

The best polymeric blank occuserts were used for incorporation of pure Levobunolol HCl drug. Calculated amount of Levobunolol HCl was dispersed in the polymeric solution and after the complete dispersion of drug, glycerine as a plasticizer was added and stirred to form a uniform dispersion. The air bubble produced during dispersion was removed by subjecting the solution for sonication. The dispersion was casted on glass plate using a ring of 4 cm diameter having (an area of 12.5714cm$^2$) 3 ml capacity[7,8]. The calculations were as follows.

### Calculation:

Diameter of the proposed occusert (d) = 0.6 cm

**Fig. 1: *In-vivo* Drug release of formulations (F2 & F1)**

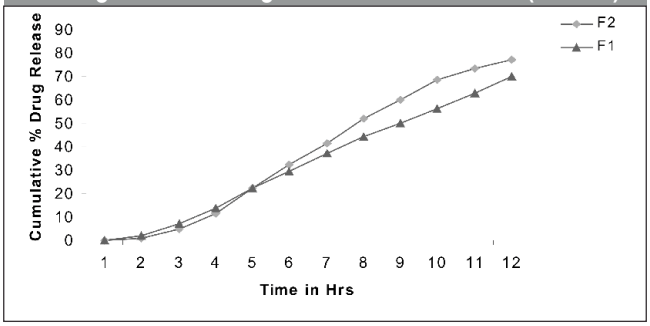


**Table 1: Compositions of different optimized Levobunolol HCl occusert formulations.**

| FC | Drug | Drug Reservoir (%) | | | | RCM (%) | |
|---|---|---|---|---|---|---|---|
| | | Film Former * | | | Plasticizer** | Film Former* | Plasticizer** |
| | Levobunolol HCl (Mg) | HPMC (%) | PVP (%) | MC (%) | Glycerine (%) | EC (%) | DBP (%) |
| $F_1$ | 0.64 | 3 | - | - | 40 | 6 | 30 |
| $F_2$ | 0.64 | 4 | - | - | 40 | 6 | 30 |
| $F_3$ | 0.64 | 3 | 1 | - | 40 | 6 | 30 |
| $F_4$ | 0.64 | 4 | 1 | - | 40 | 6 | 30 |
| $F_5$ | 0.64 | - | - | 1 | 40 | 6 | 30 |
| $F_6$ | 0.64 | - | - | 2 | 40 | 6 | 30 |
| $F_7$ | 0.64 | - | 1 | 1 | 40 | 6 | 30 |
| $F_8$ | 0.64 | - | 1 | 2 | 40 | 6 | 30 |
| $F_9$ | 0.64 | 1 | - | 1 | 40 | 6 | 30 |
| $F_{10}$ | 0.64 | 1 | - | 2 | 40 | 6 | 30 |

*Based on total volume of solvents; **Based on total polymer weight. Where, FC- Formulation code; RCM - Rate Controlling Membrane.

**Table 2: Microbial sterility testing data of best optimized Levobunolol HCl occusert formulations.**

| Samples | Bacillus Subtillis (TGM) | | | | | | | Bacteriodes Vulgate (ATGM) | | | | | | | Candida albicans (SCD) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Samples | | | | | | | | | | | | | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Control | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Unsterilized | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| F2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| F1 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - - |

TGM: Thioglycolate Medium, ATGM: Alternative Thioglycolate Medium, SCD: Soybean Casein Digest, " + " : Presence of microbial growth and " - " : Absence of microbial growth.

Radius of the proposed occusert (r) = 0.3 cm

Therefore, Area of the proposed occusert (A) = $\pi r^2$ = 0.2828 $cm^2$

Diameter of the ring (d) = 4 cm

Radius of the ring (r) = 2 cm

Total area of the ring (A) = $\pi r^2$ = 12.5714 $cm^2$

Capacity of the ring = 3 ml

No. of occusert present in the proposed area of the ring = Total area of ring / Area of one occusert = 12.5714 / 0.2828 = 44 occusert

Total amount of drug added to the polymer solution = 28.16 mg

One occusert contains = 28.16 / 44 = 0.64 mg

Amount of drug present in one occusert = 0.64 mg of Levobunolol HCl

After drying, circular films of occuserts were cut into diameter of 6 mm, each containing 0.64 mg of Levobunolol HCl and stored in an airtight container (dessicator) under ambient condition during the course of study. Finally, it is packed in a well closed light blue high intensity containers with polystyrene caps and protected from light[3, 9].

**Formulation of rate controlling membrane:**

The rate controlling membrane was casted on a glass plate using ethyl cellulose (6%), which is dispersed in chloroform and dibutyl phthalate (30 % w/w of polymer) as plasticizer. Circular membranes of 8 mm diameter were cut using a special mould[10]. Both the drug reservoir and rate controlling membrane was sealed to control release of drug from periphery.

**Animals:**

Twelve male *Orytolagus Cuniculus* healthy rabbits (2.5-3.5 kg) were used. The animals were fed with standard pellet diet and water *ad libitum* and maintained under standard environmental conditions (22 °C ± 5 °C with 12 h of light/ dark cycle). All experimental protocols were approved by Institutional Animal Ethical Committee Clearance, (601/02/c/CPCSEA), Dr. H. L. T. College of Pharmacy, Kengal, Channapatna - 571502, Bangalore (Rural), Karnataka, India.

**Sterility testing:**

Sterility testing was performed for aerobic, anaerobic bacteria and fungi by using fluid thioglycolate, alternative thioglycolate and soybean casein digest medium respectively as per the Indian Pharmacopoeia. Formulations were taken into laminar airflow and passed through a membrane filter of 0.45mm with the help of vacuum pump. After filtration, the filter paper was removed from funnel and cut into two halves. One half was dropped in bacterial media (fluid thioglycolate) and other was dropped in fungal media (soybean casein digest). Both media were kept for incubation at 37°C for 7 days and observed for any microbial growth. The sterility testing results were compared with positive and negative control samples[11].

**Modified Draize eye irritation test:**

The potential of ocular irritation and damaging effects of occuserts were evaluated by observing for any redness, inflammation and increased tear production. The rabbits were divided into two equal groups as per total number of optimized formulations. Formulations were tested on six rabbits by placing the occuserts into cul-de-sac of the left eyes, served as treated eye, while right eyes kept as control. Both eyes of the rabbits were examined for any signs of irritation at 1 hr, 24 hr and 48 hr[12].

***In-vivo* drug release studies:**

The *in-vivo* drug release studies were carried out with some modifications,[13] in which surface sterilized (using benzalkonium chloride) occuserts of formulations ($F_2$ & $F_1$) were placed in the lower eyelids of left eyes, served as treated eye, while right eyes kept as control. Eyelids were closed by

Table 3: Evaluation data of best optimized Levobunolol HCl occusert formulations by Modified Draize eye irritation test on rabbits.

| SN | Testing parts & parameters | Testing time in Hrs | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 hr | | | | | | 24 hr | | | | | | 48 hr | | | | | |
| | | Animal Code | | | | | | | | | | | | | | | | | |
| | | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI |
| **(I)** | **Cornea** | | | | | | | | | | | | | | | | | | |
| **A** | Opacity – Degree of density (area which is most dense taken for reading) | | | | | | | | | | | | | | | | | | |
| | Scattered or diffuse area – details of iris clearly visible | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Easily discernible translucent areas, details of iris slightly obscured | | | | | | | | | | | | | | | | | | |
| | Opalescent areas, no details of iris visible, size of pupil barely discernible | | | | | | | | | | | | | | | | | | |
| | Opaque, iris invisible | | | | | | | | | | | | | | | | | | |
| | Area of cornea involved | | | | | | | | | | | | | | | | | | |
| **B** | One quarter (or less) but not zero | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Greater than one quarter – less than one-half | | | | | | | | | | | | | | | | | | |
| | Greater than one-half less than three quarters | | | | | | | | | | | | | | | | | | |
| | Greater than three quarters up to whole area | | | | | | | | | | | | | | | | | | |
| | **Score = A x B x 5.** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** |
| | **Total maximum score** | **80** | | | | | | **80** | | | | | | **80** | | | | | |
| **(II)** | **Iris** | | | | | | | | | | | | | | | | | | |
| **A** | Folds above normal, congestion, swelling, circum corneal injection (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | No reaction to light hemorrhage; gross destruction (any one or all of these) | | | | | | | | | | | | | | | | | | |
| | **Score = A x 5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** |
| | **Total maximum score** | **10** | | | | | | **10** | | | | | | **10** | | | | | |
| **(III)** | **Conjunctivae** | | | | | | | | | | | | | | | | | | |
| **A** | Redness (refers to palpebral conjunctiva only) | | | | | | | | | | | | | | | | | | |
| | Vessels definitely injected above normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | More diffuse, deeper crimson red, individual vessels not easily discernible | | | | | | | | | | | | | | | | | | |
| | Diffuse beefy red | | | | | | | | | | | | | | | | | | |
| **B** | Chemosis | | | | | | | | | | | | | | | | | | |
| | Any swelling above normal (includes nictitating membrane) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Obvious swelling with partial eversion of the lids | | | | | | | | | | | | | | | | | | |
| | Swelling with lids about half closed | | | | | | | | | | | | | | | | | | |
| | Swelling with lids about half closed to completely closed | | | | | | | | | | | | | | | | | | |
| **C** | Discharge | | | | | | | | | | | | | | | | | | |
| | Any amount different from normal (does not include small amount observed in inner canthus of normal animals) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Discharge with moistening of the lids and hairs just adjacent to the lids | | | | | | | | | | | | | | | | | | |
| | Discharge with moistening of the lids and considerable area around the eye | | | | | | | | | | | | | | | | | | |
| | **Score = (A + B + C) x 2** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **6** |
| | **Total maximum score** | **20** | | | | | | **20** | | | | | | **20** | | | | | |

**Table 4: Accelerated stability study (Aging study) data of best optimized Levobunolol HCl occusert formulations.**

| Parametrs | Formulation (F2) | | | | | | | | | Formulation (F1) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Temp. in °C | | | | | | | | | Temp. in °C | | | | | | | | |
| | 4 | | | 37 | | | 50 | | | 4 | | | 37 | | | 50 | | |
| | Period (Days) | | | | | | | | | Period (Days) | | | | | | | | |
| | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 | 60 |
| **Physical Appearance** | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **Weight Variation (mg)** | 30.32 (0.06) | 30.28 (0.07) | 30.28 (0.06) | 30.33 (0.06) | 30.33 (0.06) | 30.31 (0.06) | 29.85 (0.06) | 29.90 (0.06) | 29.94 (0.06) | 28.12 (0.07) | 28.12 (0.08) | 28.10 (0.07) | 28.15 (0.08) | 28.22 (0.08) | 28.12 (0.08) | 27.76 (0.08) | 27.77 (0.08) | 27.26 (0.08) |
| **Thickness (mm)** | 0.29 (0.004) | 0.29 (0.003) | 0.29 (0.004) | 0.29 (0.004) | 0.29 (0.003) | 0.29 (0.004) | 0.39 (0.004) | 0.29 (0.003) | 0.29 (0.004) | 0.27 (0.002) | 0.28 (0.003) | 0.27 (0.003) | 0.27 (0.002) | 0.28 (0.002) | 0.27 (0.002) | 0.27 (0.002) | 0.27 (0.002) | 0.27 (0.002) |
| **Drug Content (mg)** | 0.63 (0.001) | 0.63 (0.001) | 0.62 (0.001) | 0.63 (0.001) | 0.62 (0.001) | 0.62 (0.001) | 0.62 (0.001) | 0.62 (0.001) | 0.62 (0.001) | 0.60 (0.003) | 0.60 (0.003) | 0.60 (0.002) | 0.60 (0.003) | 0.60 (0.003) | 0.60 (0.002) | 0.59 (0.003) | 0.59 (0.003) | 0.59 (0.002) |

All values are replicate of five observations. Figures inside the parenthesis indicate Standard Deviation (S.D) values. " + " : Transparent colorless.

using cotton and non-irritant adhesive tape until sampling. After 1 hr time interval, films were removed and analyzed for remaining drug content, spectrophotometrically at 258 nm up to a total period of 12 hr.

**Accelerated stability studies (Aging study):**

Accelerated stability studies of optimized formulations ($F_2$ & $F_1$) were carried out with some modifications,[14] by exposing them at 4 °C, 37 °C and 50 °C for 2 months in stability chamber. The occuserts were withdrawn at 0, 30 and 60 days intervals and tested for their physical appearance, weight variation, thickness and percentage drug content spectrophotometrically, at 258 nm.

## RESULTS AND DISCUSSION

Results of best optimized formulations ($F_2$ & $F_1$), characterized by biologically are as follows,

**Sterility testing:**

The results of sterility testing showed that, there was no evidence of microbial growth found in control and test samples as compared to unsterilized samples for aerobic, anaerobic bacteria and fungi using thioglycolate, alternative thioglycolate and soybean casein digest medium respectively. Whereas, clear macroscopic evidence of microbial growth were observed in unsterilized samples as shown in **Table-2**. Sterility testing results showed that, occusert formulations ($F_2$ & $F_1$) were passed the test for sterility.

**Modified Draize eye irritation test:**

In the development of an ophthalmic drug delivery system, assessment of eye irritation potential is an important step for measurement of ocular injury. In the Modified Draize Technique, qualitative observations of physiological effects have been transformed to quantitative measurements by assigning those numerical values, which can be arithmetically interpreted easily as shown in **Table-3**. During eye irritation study, no scattered or diffused area was observed with involvement of less than one quarter area of cornea and iris was clearly visible. Moreover, iris was found to be normal without any folds, congestion, swelling, destruction and reactivity in response to light. Furthermore, in case of conjunctivae, swelling of nictitating membrane and lids was not observed. There was also found, neither any discharge nor moistening of lids or hairs adjacent to it. Therefore, in all three sections of study at 1, 24 and 48 hr, the scores given to the rabbits were less than the total maximum scores. So, results of present study showed that, there was no irritation to the sensitive ocular tissues by the formulations ($F_2$ & $F_1$), indicating its safety to ocular treatment.

***In-vivo* drug release studies:**

I*n-vivo* drug release studies of formulations ($F_2$ & $F_1$) have been shown in **Fig. 1** by plotting graph cumulative % drug release vs time. The results of *in-vivo* drug release studies showed that, formulations ($F_2$ & $F_1$)' had released their drug content, 77.14 % and 69.83 % respectively over an extended period of 12 hr. The extended and prolonged period of drug release may be due to slow diffusion of drug from combined polymers and plasticizers and probably due to the formation of hydrogen bonds between drug and polymers, which have helped in controlling rate of drug release[15].

**Accelerated stability study (Aging study):**

Accelerated stability studies (aging study) of optimized formulations ($F_2$ & $F_1$), carried out indicated that, the ocular inserts were stable and there was no effect on the drug content for a period of 2 months, as shown in **Table-4**.

## CONCLUSION

Therefore, it can be concluded that, best optimized polymeric occuserts have appreciable strength, reliable and safe biologically. Hence, these two best optimized occusert formulations ($F_2$ & $F_1$), of Levobunolol HCl can be used as ophthalmic products in glaucoma treatment. However, these formulations are alternate to conventional eye drops to improve the bioavailability through its longer pre-corneal residence time and ability to sustain drug release. The patient compliance may be improved due to the decrease in frequency of drug administration and chances of missing the dose by the patient.

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