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**ORIGINAL ARTICLE** **OPEN ACCESS**

# Formulation Design and *In-Vitr, Ex-Vivo* and *In-Vivo* Evaluation of Ocular Insert of Dorzolamide for Glaucoma Treatment

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**ABSTRACT**

*The ocular insert have shown a potential for ophthalmic delivery over the conventional method. The objective of this research work to design controlled release ocular insert of Dorzolamide by using solvent casting technique. This technique use of HPMC K4M and PVA as film forming polymer and polyethylene glycol (PG) was used as plasticizer. Various physicochemical parameter of nine ocular insert composition evaluated including the concept in-vitro and Ex-vivo correlation studies was used in pharmaceutical research because a simple in vitro release study on a drug product will be insufficient to predict its therapeutic efficiency. The in vitro drug release study showed that the optimized ocular insert up to 6hr. Cumulative percent drug permeated from Dorzolamide ocular insert (batch F2) is 95.1%. F2 component films were free from microorganism's contamination when formulated in the sterile aseptic conditions. The Dorzolamide ocular insert formulation showed excellent ocular tolerance as determine by hen's egg test chorioallantonic membrane (HET CAM) and tolerance study (using Albino rabbit). Therefore the optimize ocular insert formulation was found to be stable, isotonic, non-toxic with higher in vitro and in vivo.*

***Keywords:*** Dorzolamide, HPMC K4M, controlled release, HET CAM, tolerance study

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## INTRODUCTION

Delivery of drug to the eye has remained as one of the most challenging tasks for pharmaceutical scientists. The intraocular bioavailability of the drug through conventional eye drops is very poor due to factors such as naso-lachrymal drainage, lacrimation, and drug dilution with tear fluid, tear turnover and conjunctival absorption.[1] 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%) drug actually penetrates the cornea and reaches the intraocular tissue [1,2,3].

A sincere attempt to prolong the contact of ophthalmic drug with cornea can improve its efficiency. This can be fulfilled by incorporating viscosity enhancing agent in eye drops or by using water insoluble ointment base in ophthalmic formulation which increase the drug content with cornea. Unfortunately, these attempts have shown limited improvement in drug cornea contact than conventional eye drop solution, but consistent drug availability is still a challenging task to be achieved to avoid repeated medication throughout the day [4]. Eye is most interesting organ due to its drug disposition characteristics. Topical application of drugs is the method of choice under most circumstances because of its convenience and safety for ophthalmic chemotherapy. A significant challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage [5].

Drugs are commonly applied to the eye for a localized action, on the surface, or in the interior of the eye. A major problem in ocular therapeutics is the attainment of an optimal drug concentration at the site of action. Poor bioavailability of drugs from ocular dosage forms is mainly due to the precorneal loss factors which include tear dynamics, non-productive absorption, transient residence time in the cul-de-sac, and the relative impermeability of the corneal epithelial membrane. Due to these physiological and anatomical constraints only a small fraction of the drug, effectively 1% or even less of the instilled dose, is ocularly absorbed. So far, attempts have been made to improve ocular drug bioavailability by extending drug residence time in the conjunctival sac and improving drug penetration across the cornea, the major pathway of drug entry into the internal eye[6].

Development of newer, more sensitive diagnostic techniques and novel therapeutic agents continue to provide ocular delivery systems with high therapeutic efficacy. Conventional ophthalmic formulations like solution, suspension, and ointment have many disadvantages which result into poor bioavailability of drug in the ocular cavity. The specific aim of designing a therapeutic system is to achieve an optimal concentration of a drug at the active site for the appropriate duration. Ocular disposition and elimination of a therapeutic agent is dependent upon its physicochemical properties as well as the relevant ocular anatomy and physiology. A successful design of a drug delivery system requires an integrated knowledge of the drug molecule and the constraints offered by the ocular route of administration [7].

Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity of front of the eye for prolong period of time. Consequently it is imperative to optimize ophthalmic drug delivery; one of the ways to do so is by addition of polymers of various grades, development of in situ gel or colloidal suspension or using erodible or non-erodible insert to prolong the precorneal drug retention [8].

## MATERIAL AND METHOD

Dorzolamide was kindly provided by Kilipch health care (Eye care) India pvt,ltd, Mumbai. PVA, PG and HPMCK4Mwas purchase from vighnesh enterprises Mumbai. All other chemical used were of reagent grade, 10 day old hen fertilized egg purchase from poultry farm (Pune, India). Albino rabbit from 1.65-1.75kg purchase from lacsmi Biofarm Alephata, Junnar, Pune.

### Formulation of Dorzolamide Ocular inserts

Solvent casting technique was used to prepared ocular insert. The required quantity of polymer is dissolved in 30ml of distilled water and stirred for 2hr then weighed quantity of Dorzolamide was added and stirred for 2hr on magnetic stirrer to get a uniform dispersion. After complete mixing casting solution was poured on petridish and allowed it to dry. The dried inserts thus obtained were cut into required size and wrapped in aluminum foil and stored. Composition of different batches

### Evaluation of Dorzolamide Ocular inserts

#### A. Thickness

Inserts thickness was determined using a caliper (Digital vernier caliper) and recorded as the mean of measurements [7].

#### B. Folding Endurance

Folding endurance was determined by repeatedly fold the film at the same place till breaking or first sign of breaking. The number of time the film could be folded at the same place without breaking gives the folding endurance value [7].

#### C. Surface pH

The Dorzolamide inserts were allowed to swell in closed petridish at room temperature for 30 min in 1 ml of distilled water. The swollen device was removed and solution placed under digital pH meter to determine the surface pH [8].

#### D. Weight Uniformity

From each batch (n = 3), inserts were taken and weighed individually using digital balance. The mean weights of the insert were recorded. [7]

#### E. Drug Content Uniformity

To check the uniformity of drug in insert, three inserts were taken from each formulation. Each insert was placed in a glass vial containing 10 ml of artificial tear fluid. The insert was dissolved by aid of a magnetic stirrer, solution was then filtered and 1 ml from filtrate was withdrawn and diluted up to 10 ml distilled water and absorbance was measured by UV–Visible spectrophotometer at 253.5 nm [7].

#### F. Tensile strength

Tensile strength of the prepared films was calculated according to the following equation. [9, 10, 11, 12]

$$\text{Tensile strength} = \frac{\text{N}}{\text{mm}^2}$$

i. e.

$$\frac{\text{Breaking load N}}{\text{Cross sectional area of the sample mm}^2}$$

### Drug release study

*In vitro* drug release from the different ocular inserts was studied by using franz diffusion cell and dialysis membrane. The dialysis membrane mimics corneal epithelium. The receptor compartment was filled with freshly prepared 50ml artificial tear fluid pH-7.4(ATF). 1.5 $cm^2$ area of ocular film was placed on the dialysis membrane and opening of the donor compartment was sealed with a glass cover slip, while the receptor fluid was maintained at 37 ± 0.5ºC with constant stirring, using magnetic stirrer. 1 ml sample

was withdrawn from receptor compartment at various time intervals up to 6 h and was analyzed spectrophotometrically at 253.5 nm. Each sample withdrawn was replaced with equal volume of artificial tear fluid [13].

***Ex vivo* transcorneal permeation study**

Whole eye ball of goat was transported from local butcher shop to the laboratory in cold (4ºC) normal saline within 1 h of slaughtering the animal. The cornea was carefully excised along with 2–4 mm of surrounding scleral tissue and was washed with cold normal saline till the washing was free from proteins. Isolated cornea was mounted by sandwiching surrounding scleral tissue between clamped donor and receptor compartments of an all glass modified Franz diffusion cell in such way that its epithelial surface faced the donor compartment. The receptor compartment was filled with freshly prepared artificial tear fluid. 1.5cm$^2$ area of ocular film was placed on the cornea and opening of the donor compartment was sealed with a glass cover slip, while the receptor fluid was maintained at 37 ± 0.5ºC with constant stirring, using magnetic stirrer. 1ml sample was withdrawn from receptor compartment at various time intervals up to 6 h and was analyzed spectrophotometrically at 253.5 nm. Each sample withdrawn was replaced with equal volume of artificial tear fluid [9].

**Sterility testing as per I.P. 2014**

The test for sterility on the sterilized ocular insert was carried out by direct inoculation method [14, 15].

**Culture media**

Alternate thioglycolate medium and soyabean casein digest medium was used as a culture medium for bacteria (*S. aureus*) and fungi (*C. albicans*) respectively. Media were prepared according to I.P.2014 and 20 ml was taken in boiling test tube, properly plugged with cotton and sterilized by autoclaving at 121ºC at 15 lb/inch gauge pressure for 20 minutes.

**Inoculation and incubation**

Formulation was aseptically added in test tube containing respective media and simultaneously positive and negative control was prepared for each media. The inoculated culture media for bacteria and fungi were incubated at 30$^o$C - 35$^o$C and 20$^o$C - 25$^o$C respectively in incubator for not less than 14 days [16].

**Accelerated stability studies as per ICH Guidelines**

Stability studies for the optimized batch F2 was carried out to determine the effect of presence of formulation additives on the stability of the drug and also to determine the physical stability of the formulation under accelerated storage conditions.The optimized batch F2 was subjected to elevated temperature and humidity conditions of 25±1°C/ 60%RH, 30±1°C/ 65%RH and 40 ± 2$^o$C/ 75 ± 5 % RH. Samples were withdrawn at the end of 0, 30, 60 and 90 days and evaluated for physical appearance, and drug content [17].

**HET CAM Study**

HET CAM (Hen's egg chorioallantoic membrane) study is an substitute to the Draize in vivo rabbit eye test for the recognition of ocular irritations [18]. The hen's egg chorioallantoic membrane bioassay was accomplished using 10 day fertilized eggs. Prior to use, the eggs were candled to notice the viability of the embryo. Dorzolamide Solution was comparatively studied with commercial formulation. Experiments were performed in triplicates using sodium chloride as a negative control and sodium hydroxide as a positive control. The CAM was treated with 500 µl of the sample and irritation levels were checked by observing for signs of irritation such as hemorrhage, lysis and coagulation at different time intervals up to 5 min. Potential irritation scores (PIS) were calculated by the formula given below:

$$\text{PIS} = \frac{(301 - \text{h})}{300} \times 5 + \frac{(301 - \text{l})}{300} \times 5 + \frac{(301 - \text{c})}{300} \times 9$$

Where h is the time in seconds when haemorrhage appears; l is the time in seconds when lysis appears; c is the time in seconds when coagulation appears.

**Ocular *In-Vivo* Tolerance Studies**

The rabbits were randomly divided into three groups (Three rabbits per group). Three groups of 9 male New Zealand rabbits each were made and were marked as test, positive control and negative control respectively and kept in separate cage. The negative control group received 0.9% NaCl, the test group received sterile best formulation and positive control group received standard marketed formulation (Eye Drop) 2-3 drops of test solution was normally placed in the lower cul-de-sac once a day for a period of 7 days and irritancy was tested at the time interval of 1 hr, 24 hr, 72 hr and 1 week after administration. The rabbits were observed periodically for redness, swelling and watering of the eye.

Ocular inserts (F2) were selected for in vivo study. The first group (group I) received the normal saline solution the second group (group II) received the Dorzolamide ocular inserts belonging to F2, However, the third group (group III) received the Dorzolamide solution (Eye Drop Marketed Formulation). Each investigated ocular insert formulation (50µl) was placed in the lower cul-de-sac of rabbit's right eye, (Fig.

9). Subsequently, the ocular condition of both eyes of the rabbits were visually evaluated by examining the following parameters: redness, inflammation, surface of the cornea, and tear production using a slit lamp immediately after treatment and at 0.5, 1, and 2h after insert application. The degradation and disappearance of the inserts was recorded by lifting the lower eyelid to determine their biodegradability. Moreover, the general behavior of the rabbits was also monitored. All observations were made by two independent operators [19].

## RESULTS AND DISCUSSIONS

Ocular insert was prepared by solvent casting method and further evaluated.

**Table 01: Composition of different batches of Dorzolamide ocular insert**

| Name of Ingredients (mg) | Different Batches of Dorzolamide ocular insert | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
| PVA | 700 | 700 | 700 | 700 | 700 | 700 | 700 | 700 | 700 |
| HPMC K4M | 20 | 20 | 20 | 40 | 40 | 40 | 60 | 60 | 60 |
| PG | 144 | 180 | 216 | 148 | 185 | 222 | 152 | 190 | 228 |
| Dorzolamide | 265 | 265 | 265 | 265 | 265 | 265 | 265 | 265 | 265 |
| Water(up to ml) | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |

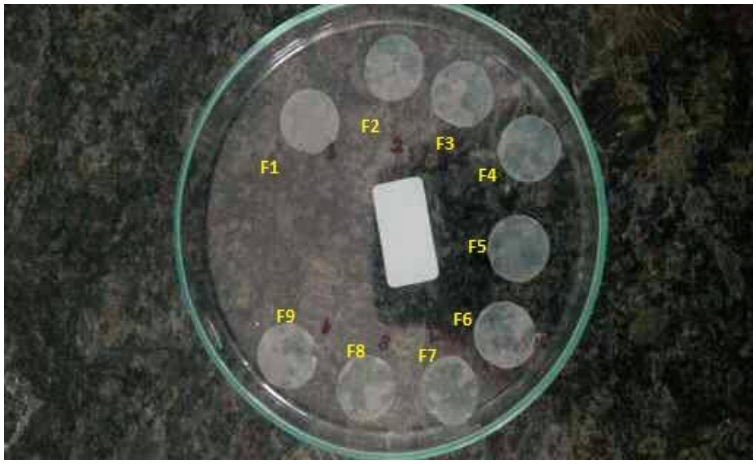



**Fig. No 01:- Formulation of Dorzolamide**

### Thickness Measurement

Inserts thickness was determined using a calliper (Digital caliper vernier) and recorded as the mean of measurements. Table 02 Indicates that prepared films were uniform in thickness which is found to be directly related to concentration of polymer.

### Folding Endurance

The folding endurance of films were recorded which reflects the flexibility of films. This test ensures that the prepared films were suitable to produce continuous film without breaking or tearing. The respective results are given in Table 02. From obtained results it was observed that all film formulation was having considerable flexibility.

### Surface pH

The developed formulation should be non irritant after administration. The surface pH of the prepared ocular insert was found to be in range of 6.6 ± 0.02 to 6.8 ± 0.04 indicating safe in chronic treatment of eye infection as shown in Table 02.

### Weight Uniformity

The weights of the Dorzolamide ocular inserts were found to be in the range of 2.2 ± 0.05 to 2.8 ± 0.2 mg (Table 02). The uniformity of weights of films indicates good distribution of drug in polymer and plasticizer.

### E. Drug Content Uniformity

Drug content of Dorzolamide ocular insert were determined and it was found to be in between 97.89-98.76% of Dorzolamide given in (Table 02) the results indicated that drug was uniformly dispersed.

**Table 02: Physicochemical evaluation of ocular inserts**

| Different Batches | Evaluation Parameters | | | | |
|---|---|---|---|---|---|
| | Thickness (mm) | Folding Endurance | Surface pH | Weight Uniformity (mg) | Drug Content (%) |
| F1 | 0.10 | 209 ± 1.2 | 6.6 ± 0.06 | 2.3 ± 0.05 | 98.35 ± 0.08% |
| F2 | 0.11 | 222 ± 2.0 | 6.6 ± 0.06 | 2.2 ± 0.05 | 98.51 ± 0.03% |
| F3 | 0.10 | 226 ± 0.6 | 6.6 ± 0.06 | 2.3 ± 0.05 | 98.36 ± 0.03% |
| F4 | 0.11 | 263 ± 2.6 | 6.7 ± 0.05 | 2.4 ± 0.05 | 97.89 ± 0.07% |
| F5 | 0.12 | 282 ± 1.7 | 6.6 ± 0.06 | 2.5 ± 0.06 | 98.57 ± 0.06% |
| F6 | 0.11 | 275 ± 3.0 | 6.7 ± 0.05 | 2.4 ± 0.05 | 97.92 ± 0.04% |
| F7 | 0.12 | 318 ± 2.6 | 6.8 ± 0.06 | 2.6 ± 0.06 | 98.76 ± 0.04% |
| F8 | 0.12 | 332 ± 2.0 | 6.7 ± 0.05 | 2.6 ± 0.11 | 98.19 ± 0.06% |
| F9 | 0.11 | 326 ± 4.2 | 6.8 ± 0.06 | 2.8 ± 0.21 | 98.57 ± 0.06% |

**Tensile Strength**

Tensile strength of the film was checked by universal tensile strength testing machine. Tensile strength of Dorzolamide ocular insert was found to be 51.45 gm/mm, which means formulation showed good mechanical strength so as to withstand any damage while handling or during transportationFig. 02.

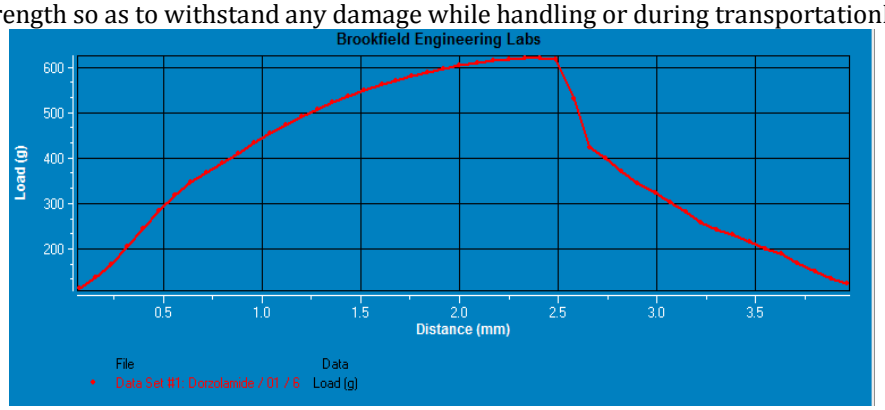



**Figure 02: Tensile Strength of optimized batch (F2)**

***In Vitro* Drug Release Study**

The *in vitro* permeation studies of dorzolamide ocular inserts were carried out through the dialysis membrane clamped between donor and receptor compartment of Franz diffusion cell. The result indicates that as the concentration of HPMC K4M is increases the release of drug is decreases. HPMC K4M acts as a release retardant. The results are shown in figure 03 which indicates that the *in vitro* drug release from ocular inserts (F1 to F9) was sustained for 6 h. F2 batch was found to be more desired than other batches.

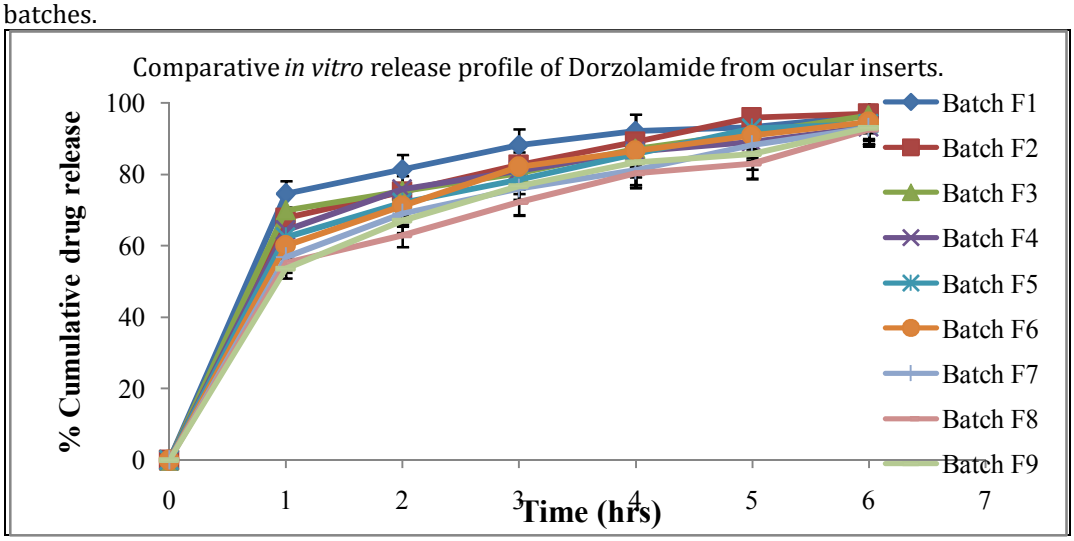



**Figure 03: *In vitro* release profile of all 9 formulations of Dorzolamide ocular inserts.**

**Analysis of Data by Design-Expert**

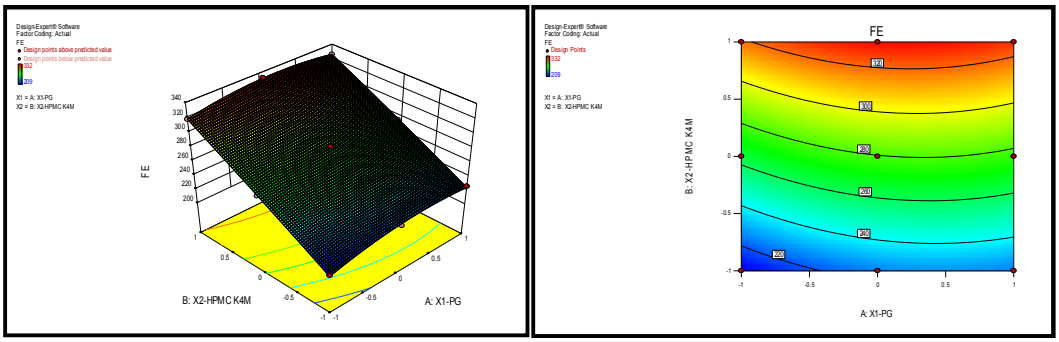
All the responses observed for different formulations prepared were fitted to various models using Design-Expert Software.

**Response surface plots analysis and Contour plots**

**Effect of formulation variable on folding endurance**

The model for response Y1 (Folding Endurance) is as follows:

$Y_1(FE) = +279.44+6.16X_1+53.16X_2-2.25X_1X_2-9.16\ X_1^2-1.16\ X_2^2$ (1)




(a) (b)

**Figure 04: a) 3D Surface plot b) Contour plot for Folding Endurance**

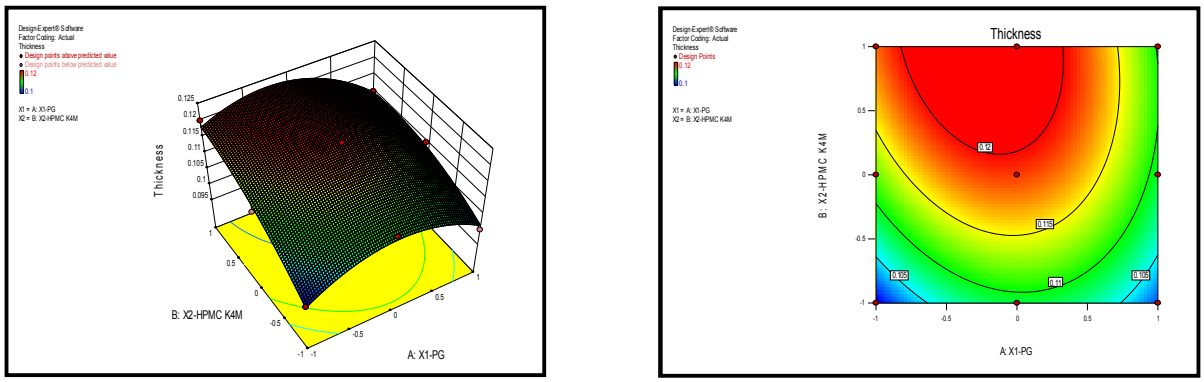
Above equation (1) indicates that ($X_1$) Concentration of PG and ($X_2$) Concentration of HPMC K4M, both have significant effect on folding endurance. Effect of (X1) and (X2) can be further explained by response surface plots and contour plots. (Figure 04).

**Effect of formulation variable on thickness**

The model for response Y2 (Thickness) is as follows:

$Y2\ (Thickness) = +0.12-1.7X_1+6.66X_2-2.5X_1X_2-8.33X_1^2-3.33X_2^2$ (2)

Above equation (2) indicates that ($X_1$) Concentration of PG and ($X_2$) Concentration of HPMC K4M, Both have significant effect on Thickness. Effect of ($X_1$) and ($X_2$) can be further explained by response surface plots and contour plot no 5.



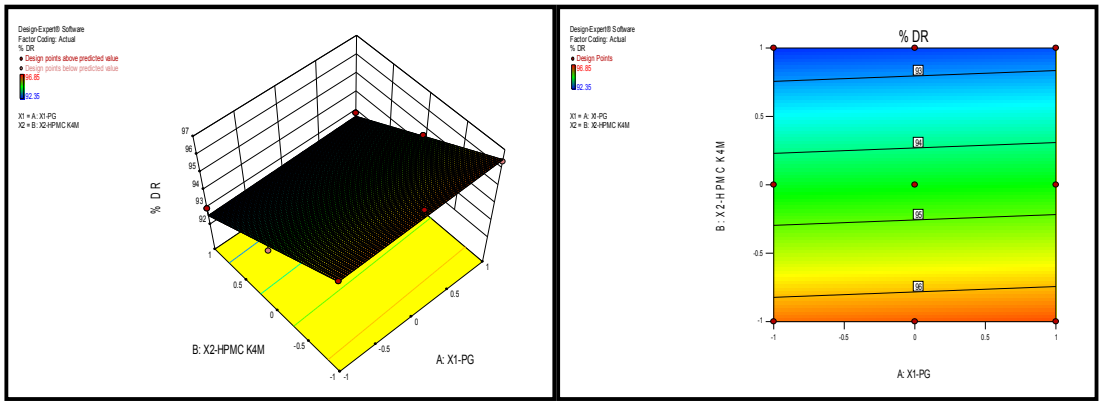

(a) (b)

**Figure 05: a) 3D Surface plot b) Contour plot for Thickness**

**Effect of formulation variable on drug diffusion**

$Y3\ (\%\ Drug\ Release) = +94.51+0.07*X_1-1.90*X_2$ (3)

Above equation (3) indicates that ($X_1$) Concentration of PG and ($X_2$) Concentration of HPMC K4M. Both have significant effect on % Drug Release. Effect of ($X_1$) and ($X_2$) can be further explained by response surface plots and contour plots. (Figure 06)




(a) (b)

**Figure 06: a) 3D Surface plot b) Contour plot for % Drug Release**

***Ex vivo* trans corneal permeability study:**

Drug candidate must possess adequate permeability to be delivered successfully through ophthalmic route. Physicochemical properties of drug plays key role in permeability. Goat cornea was used for *ex vivo* trans corneal permeability study of Dorzolamide ocular insert. Cumulative percent drug permeated from Dorzolamide ocular insert (batch F2) is 95.1% which is shown in Figure 07.

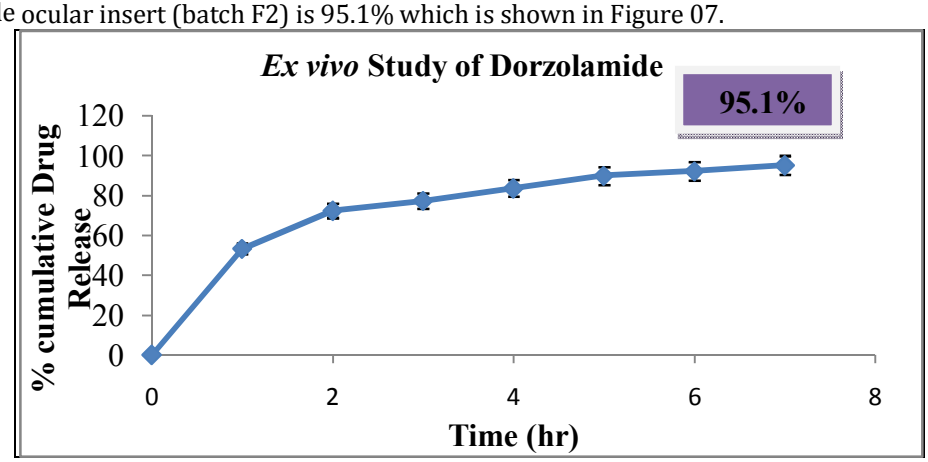



**Figure 07: *Ex vivo* transcorneal cumulative % drug release**

**Sterility Testing as per IP 2014**

In this study the presence of microorganisms is checked after sterilization. Ocular inserts were sterilized by gamma radiation. For this study alternative thioglycolate medium(ATGM) was used to analyze presence of bacteria and Soybean case in digest medium (SBCD) was used for presence of fungi. The turbidity occurring in the media indicates presence of microbes. Optimized batch component films were used to perform sterility testing and results are given in Table 03 and Figure 08.

**Table 03: Sterility Testing for Batch F2**

| No. | Medium | Days | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1. | Alternative Thioglycolate Medium | 1 | 2 | 3 | 4 | 5 | 10 | 14 |
| | Control | - | - | - | - | - | - | - |
| | Sterilized film | - | - | - | - | - | - | - |
| | Unsterilized film | - | + | + | + | + | + | + |
| | Positive | - | + | + | + | + | + | + |
| 2. | Soyabin Casein Digest Medium | | | | | | | |
| | Control | - | - | - | - | - | - | - |
| | Sterilized films | - | - | - | - | - | - | - |
| | Unsterilized films | - | + | ++ | + | ++ | + | + |
| | Positive | - | + | + | + | + | + | + |

In above table (-) sign indicates no growth of microorganisms and (+) sign indicate the growth of microorganisms. Optimized batch F2 component films were free from microorganism's contamination when formulated in the sterile aseptic conditions.

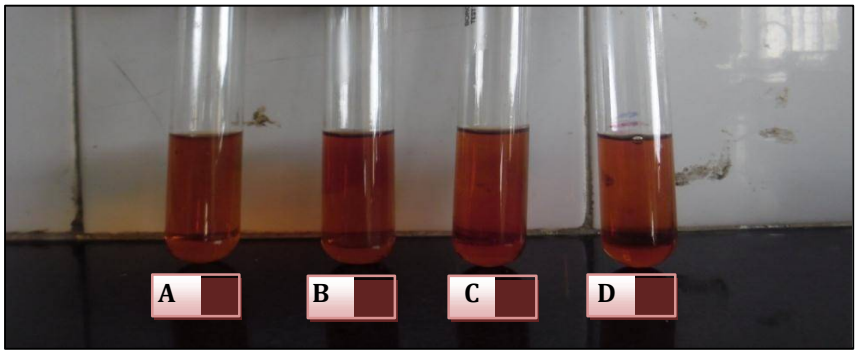



**Figure 08: Sterility testing**

In figure- 8 (A) indicates Positive, (B) indicates unsterilized films, (C) indicates Sterilized films, and (D) indicates as control.

**K. Accelerated Stability Studies:**

Stability study was carried out on optimized ocular inserts formulation for three months. It was found that formulation remained stable at various conditions of temperature and relative humidity used as per ICH guidelines. The results obtained are shown in Table 09. The results showed that there was no change in physical appearance of ocuserts, Drug content showed no marked change after three months. These results concluded that ocuserts were chemically and physically stable at different temperature and humidity conditions for three months.

**Table 09: Stability Study Data**

| Time in Month | Temperature / %RH | Parameters | |
|---|---|---|---|
| | | Appearance | % Drug content |
| 1 | 40 ± 2ºC/ 75 ± 5 | No change | 98.95 |
| 2 | 40 ± 2ºC/ 75 ± 5 | No change | 98.87 |
| 3 | 40 ± 2ºC/ 75 ± 5 | No change | 98.79 |

**HET CAM Study**

Optimized formulation was subjected to HET CAM study. Previous reports suggest that there is good correlation between HET-CAM study and Draize eye test. HET-CAM study depends upon discrete elucidations. The score was recorded for HET CAM study as per the below mentioned chart. The average score was obtained as 0 for group-I received normal saline solution and group-II treated with D-ocular-insert (F2). Hence both found to be non-irritant. This study reveals that the D-ocular insert is non-irritant and tolerable shown in table 10.

**Table 10: Standard HET-CAM score followed as evaluation parameter for Ocular Tolerance**

| Sr. No. | Effect of Formulation on CAM | Score | Presumption |
|---|---|---|---|
| 01. | No visible haemorrhage | 0 | No irritation |
| 02. | Observable membrane discoloration | 1 | Mild- Irritant |
| 03. | Structure cover partially the area of CAM due to membrane discoloration | 2 | Moderate Irritant |
| 04. | Structure fully cover he area of CAM due to membrane discoloration | 3 | Severe Irritant |

***In-vivo* Tolerance Study:**

The clinical acceptability of topically applied ocular inserts may be limited by their annoying ocular adverse effects, such as, irritation, burning, stinging, and tearing, that may provide a reason for patients to stop their medication. In general, the ocular inserts must be well tolerated in the eyes and should not cause any irritation or inconvenience to the patients. Accordingly, the potential ocular adverse and/or damaging effects of the ocular inserts under investigation were evaluated by observing the conjunctiva and cornea of rabbits' eyes at specific time intervals after ocular insert administration. It is worth mentioning that during and after ocular administration of different treatments, all animals were calm and did not show any signs of discomfort except for the animals belonging to group I that were treated using Saline Solution. These animals were slightly agitated and showed an increase in reflex blinking which was related to ocular discomfort. However, their intake of food and water was normal during the study.

Initial mild redness and lacrimation that lasted for less than 1 min was observed in rabbit's eye (group II) after direct instillation of Dorzolamide insert. This is expected to cause rapid flushing of the drug into the Nasolacrimal gland with a probable decrease in the ocular efficacy. Regarding the Dorzolamide ocular inserts (F2) applied in the lower cul-de-sac of rabbits belonging to group II respectively; they were in general well tolerated and didn't show any visible redness or inflammation in the conjunctiva and cornea of rabbit's eyes. This observation suggests the absence of any irritation potential associated with their ocular administration. However, minimal lacrimation without any redness occurred immediately after application of the previously mentioned ocular inserts. This was extremely advantageous as it initiated their rapid hydration and softening followed by their adherence on to the application site.

**Normal Group-I** **D-Occular insert Group-II** **D- Solution Group-II**

**Figure 09:.*In-vivo* Tolerance Study**

**Table 11: Grading for Rabbit Eye irritation Study**

| Grading for Rabbit Eye irritation Study | |
|---|---|
| **Observation** | **Score** |
| **REDNESS** | |
| Normal | 0 |
| Some Blood vessel Hyperaemic | 1 |
| Diffuse and Carmison Colour | 2 |
| Beefy red | 3 |
| **INFLAMMATION** | |
| Normal | 0 |
| Mild | 1 |
| Moderate | 2 |
| Sever | 3 |
| **SWELLING** | |
| Normal | 0 |
| Some swelling Above Normal | 1 |
| Partial Eversion of Lid | 2 |
| Some swelling with half closed lid | 3 |
| **TEAR PRODUCTION** | |
| Normal | 0 |
| Low Lacrimation | 1 |
| Medium Lacrimation | 2 |
| High Lacrimation | 3 |

**Table 12: *In-Vivo* Tolerance Study**

| *In-Vivo* Tolerance Study | | | | | |
|---|---|---|---|---|---|
| **Group** | **Treatment** | **Parameter** | **0.5 Hrs** | **1 Hrs** | **2 Hrs** |
| **01** | Normal Saline Solution (3 Animal) | Redness | 1.333 ± 0.577 | 0.333 ±0.577 | 0 |
| | | Inflammation | 0.333 ±0.577 | 0 | 0 |
| | | Tear Production | 1.666 ±0.577 | 0 | 0 |
| **02** | Dorzolamide Ocular Insert (3 Animal) | Redness | 2 ±1 | 0.333 ±0.577 | 0 |
| | | Inflammation | 0 | 0 | 0 |
| | | Tear Production | 1 ±0 | 0.333 ±0.577 | 0 |
| **03** | Dorzolamide Eye Drop (3 Animal) | Redness | 2.666 ±0.577 | 2.333 ±0.577 | 0 |
| | | Inflammation | 0 | 0 | 0 |
| | | Tear Production | 2.666 ±0.577 | 2.666 ±0.577 | 0 |

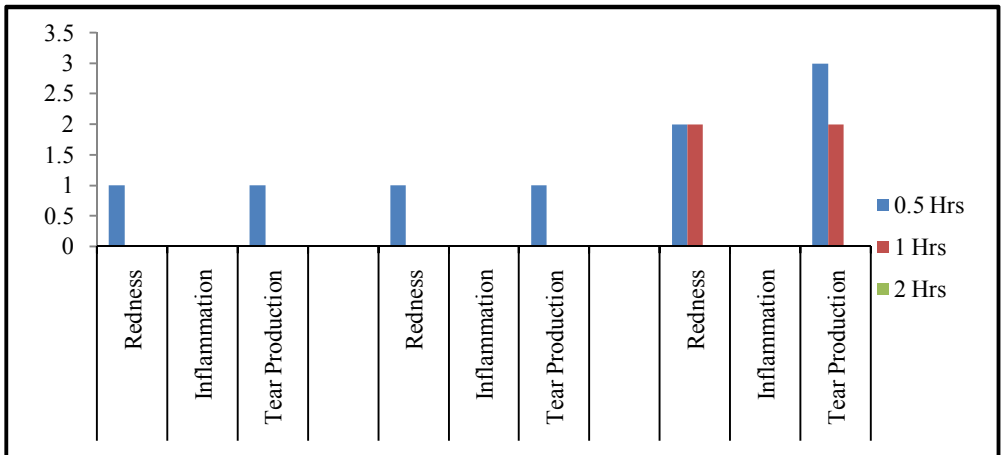



**Figure 10: Graph of *In-Vivo* Tolerance Study**

## CONCLUSION

Method of preparation of ocular inserts was found to be simple and reproducible. The polymers used were non-toxic, relatively less expensive and easily available. Polymers were found to be effective at different concentration in providing a constant release of drug from the formulation for a longer period of time.

## STATEMENT OF HUMAN AND ANIMAL RIGHTS

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study. All institutional and national guidelines for the care and use of laboratory animals were followed.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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