

## FORMULATION AND DEVELOPMENT OF CONTROLLED RELEASE OCULAR INSERTS OF TIMOLOL MALEATE AND DORZOLAMIDE HYDROCHLORIDE FOR TREATMENT OF GLAUCOMA

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### KEYWORDS

Glaucoma, Timolol maleate, Dorzolamide hydrochloride, HPMC k4m, Controlled release

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### Abstract

The goal of present investigation was to formulate and evaluate ocular insert of Timolol maleate and Dorzolamide hydrochloride for the treatment of glaucoma. Glaucoma is a chronic progressive ocular disorder characterized by optic nerve damage and visual loss with increased intraocular pressure i.e. IOP is a major risk factor. The insert of timolol maleate and dorzolamide hydrochloride were prepared by using different polymers HPMC K4M, propylene glycol, polyvinyl alcohol by solvent casting method with an objective reducing frequencies of administration, increasing contact time, achieving controlled release and improving therapeutic efficacy. The formulations were evaluated for physical characteristics, thickness, folding endurance, drug content, surface pH, swelling, in-vitro drug release, sterility, stability and ocular tolerance. Drug content ranged from approximately 96.25% to 98.95%, with F3 showing  $98.95 \pm 0.03\%$ . The goat cornea was used for Ex-vivo trans corneal permeability study of timolol maleate and dorzolamide ocular insert. The stability study was carried out for three months of optimized ocular insert. Then the inserts were sterilized by gamma radiation. For this study alternative thioglycolate medium and soyabean casein digest medium were used to analyse presence of bacteria and fungi.

### Introduction:

Glaucoma is a chronic, progressive eye disorder characterized by damage to the optic nerve and irreversible loss of vision. Elevated intraocular pressure (IOP) is the major modifiable risk factor for glaucoma. The disease is often asymptomatic in its early stages, making early diagnosis and treatment essential. Treatment mainly aims to reduce IOP using drugs such as

prostaglandin analogues,  $\beta$ -blockers, and carbonic anhydrase inhibitors. Dorzolamide hydrochloride decreases aqueous humor production by inhibiting carbonic anhydrase, while timolol maleate reduces aqueous humor formation through  $\beta$ -adrenergic blockade. Conventional eye drops have limitations including rapid tear drainage, poor ocular bioavailability, frequent dosing, and reduced patient compliance. Therefore, ocular inserts are

being investigated as an alternative drug-delivery system capable of prolonging ocular residence and providing controlled drug release. A dorzolamide–timolol ocular insert may consequently improve therapeutic effectiveness and patient convenience in long-term glaucoma management.[1]

### Materials and methods:

Timolol maleate and Dorzolamide hydrochloride were obtained as gift samples from Ven Petrochem and Pharma (India) Pvt Ltd and Hetro Drug Limited. HPMC K4M, propylene glycol, polyvinyl alcohol was provided as gift samples from Shree Sadguru Hitechlab, Pune. All other chemicals and solvents used were of analytical grade. The absorption maxima of Timolol Maleate and Dorzolamide

Hydrochloride at 295 nm and 254 nm, by using UV-Visible spectrophotometer (UV-1700, Shimadzu, India). The compatibility study between timolol maleate, dorzolamide hydrochloride and polymers were ruled out using FTIR (Bruker) and DSC (Shimadzu, Japan).[2]

### Preparation of ocular insert:

Solvent casting method 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 Timolol Maleate and Dorzolamide hydrochloride was added and stirred for 2hr on magnetic stirrer to get a uniform dispersion. After complete mixing casting solution was poured on Petri dish and allowed it to dry the dried inserts thus obtained were cut into required size and wrapped in aluminium foil and stored.[3]

**Table 1: Composition of different batches of Timolol Maleate and Dorzolamide Hydrochloride ocular insert**

| Name of Ingredients (mg)  | Different Batches of Timolol Maleate and Dorzolamide ocular insert |     |     |     |     |     |     |     |     |
|---------------------------|--------------------------------------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
|                           | F1                                                                 | F2  | F3  | F4  | F5  | F6  | F7  | F8  | F9  |
| Dorzolamide Hydrochloride | 20                                                                 | 20  | 20  | 20  | 20  | 20  | 20  | 20  | 20  |
| Timolol Maleate           | 5                                                                  | 5   | 5   | 5   | 5   | 5   | 5   | 5   | 5   |
| Polyvinyl Alcohol         | 500                                                                | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 |
| HPMC K4M                  | 20                                                                 | 20  | 20  | 40  | 40  | 40  | 60  | 60  | 60  |
| Propylene Glycol          | 144                                                                | 180 | 216 | 148 | 185 | 222 | 152 | 190 | 228 |
| Water (up to ml)          | 30                                                                 | 30  | 30  | 30  | 30  | 30  | 30  | 30  | 30  |

### Optimization of Ocular insert

For optimization of Timolol Maleate and Dorzolamide Ocular insert, 32 randomized full

factorial design was selected. The design was applied to study the effect of concentration of HPMC K4M and Propylene Glycol on

formulation. The amount (%) of plasticizer, Propylene Glycol (X1) and the amount (%) of polymer, HPMC K4M (X2) were selected as independent variables, in this study. These two factors were evaluated at 3 levels as higher, middle and lower levels with coding +1, 0 and -1 respectively. Levels of X1 20%, 25%, and 30% The dependent or response variables included Thickness (Y1), Folding Endurance (Y2), cumulative % drug Released (Y3).[4]

### Evaluation of ocular insert:

The evaluation of ocular inserts were evaluated for the following parameter as described below.

**A. Visual examination:** The prepared ocuserts were visually examined for their transparency, entrapped air bubbles, color homogeneity, and any other defects.[5]

**B. Thickness:** The prepared ocuserts were evaluated for their thickness uniformity after being dried at 60°C for four hours. Ocuserts thickness was measured using micrometer Film thickness was measured for three ocuserts from each batch and the mean values as well as the  $\pm$ SD were calculated.[5]

**C. Folding Endurance:** The folding endurance of the prepared films is expressed as the number of times the insert can be folded at the same place without breaking or developing a

visible crack. The ocusert was folded at the center, between the index finger and the thumb and then opened. This was termed as one folding. This process was repeated until the ocusert show a breakage or cracks in the center; the number of folds was counted for each formula. This experiment was repeated three times for each formula and the mean values  $\pm$ SD were calculated.[4,5]

**D. Weight Uniformity:** The prepared ocuserts were dried at 60°C for four hours and five different ocuserts from each prepared formula were weighed individually. The average weight of formula and standard deviation ( $\pm$ SD) was calculated. The mean weights of the insert were recorded.[4,5]

**E. Drug Content Uniformity:** Five ocuserts from each formula were taken separately and dissolved by means of magnetic stirring in 10 ml STF pH 7.4, the solutions were filtered through filter papers and each solution was completed to 50 ml with Simulated tears fluid. The amounts of both Timolol Maleate and Dorzolamide were determined for each solution using the fore-mentioned HPLC method. Mean value and  $\pm$ SD were calculated for each formula.

**F. Percentage moisture absorption Determination:** Three inserts were kept

in a desiccator at room temperature for 24 hrs. containing calcium chloride. They were then taken out; initial weight was taken and exposed to 75% relative humidity using a saturated solution of sodium chloride in desiccator. These inserts were weighed repeatedly until they showed a constant weight. Percent moisture uptake was calculated using the following formula:

$$\text{Percentage moisture absorption} = \frac{[(\text{Final weight} - \text{Initial weight}) / \text{Initial weight}] \times 100}{100}.$$

**G. Surface pH:** Agar (2% m/v) was dissolved in warm simulated tear fluid (STF), pH 7.4 under stirring and then poured the solution into petri plates, allowed till gelling at room temperature. Inserts were left to swell for 5 h on agar plate. The surface pH was measured by means of a pH paper placed on the surface of swollen film.

**H. Swelling index determination:** Three inserts were weighed and placed separately in beakers containing 4 ml of distilled water. At regular time intervals, inserts were removed and the excess water on their surface was wiped using a blotting paper and again weighed. The procedure was continued till there was no increase in the weight of inserts. Swelling index was then calculated by dividing the increase in weight by the

original weight and was expressed as percentage.

**I. *In vitro* 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 artificial tear fluid. 1.5 cm<sup>2</sup> 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  $37 \pm 0.5^\circ\text{C}$  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. Each sample withdrawn was replaced with equal volume of artificial tear fluid.

**J. *Ex vivo* trans corneal permeation study:** Whole eye ball of goat was transported from local butcher shop to the laboratory in cold ( $4^\circ\text{C}$ ) 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<sup>2</sup> 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  $37 \pm 0.5^\circ\text{C}$  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. Each sample withdrawn was replaced with equal volume of artificial tear fluid.[5]

#### K. Drug release kinetics

Drug release mechanisms and kinetics are the two important characteristics of a drug delivery system in describing drug dissolution profile. To describe the kinetics of the drug release from optimized Ocular insert, mathematical models such as zero-order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas models were used. The criterion for selecting the most appropriate model was chosen on the basis of the goodness or fit test.[6]

1. Zero-order Kinetic model – Cumulative %drug release versus Time.
2. First-order Kinetic model – Log cumulative % drug remaining versus Time.
3. Higuchi's model – cumulative percent drug released versus square root of time.
4. Korsmeyer equation / Peppas's model- Log cumulative percent drug released versus log time.

#### 1. Zero order kinetic: It describes the system in which the release rate is independent of its concentration

$$Q_t = Q_0 + K_0t$$

Where,

$Q_t$  = amount of drug dissolved in time  $t$   $Q_0$  = initial amount of drug in the solution

$K_0$  = zero order release constant

If the zero order drug release kinetic is obeyed, a plot of  $Q_t$  versus  $t$  will give straight line with a slope of  $K_0$  and an intercept at  $Q_0$ .

#### 2. First Order Kinetic

It describes the drug release from the system in which the release rate is concentration dependent.

$$\text{Log } Q_t = \text{log}Q_0 + K_1t/2.303$$

Where  $Q_t$  = amount of drug dissolved in time

$Q_0$  = initial amount of drug in the solution

$K_1$  = first order release constant

If the release pattern of drug follows first order kinetics, then a plot of  $\log (Q_0 - Q_t)$  versus  $t$  will be straight line with a slope of  $K_1 / 2.303$  and an intercept at  $t = 0$  of  $\log Q_0$ .

### 3. Higuchi's Model

It describes the fraction of drug release from a matrix is proportional to square root of time.

$$M_t / M_\infty = KHt^{1/2}$$

Where,

$M_t$  and  $M_\infty$  are cumulative amount of drug release at time  $t$  and infinite time, and  $KH$  = Higuchi dissolution constant reflection formulation characteristics.

If the Higuchi model of drug release is obeyed, then a plot of  $M_t / M_\infty$  versus  $t^{1/2}$  will be straight line with slope of  $KH$ .

### 4. Korsmeyer-Peppas Model

The power law describes the fractional drug release is exponentially related to the release time and adequately describes the release of drug from slabs, cylinders and spheres, as expressed in following equation.

$$M_t / M_\infty = K t^n$$

$$\text{Log } (M_t / M_\infty) = \log K + n \log t$$

**Table No.2: Mechanism of Drug Release as per Korsmeyer Equation/ Peppas's Model**

| Sr. No. | 'n' value         | Drug release            |
|---------|-------------------|-------------------------|
| 1       | 0.45              | Fickian release         |
| 2       | $0.45 < n < 0.89$ | Non-Fickian release     |
| 3       | 0.89              | Case II transport       |
| 4       | Higher than 0.89  | Super case II transport |

#### L. Sterility testing as per I.P. 2014

The test for sterility on the sterilized ocular insert was carried out by direct inoculation method.

#### Culture media

Alternate thioglycolate medium and soybean 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 121°C 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°C - 35°C and 20°C

- 25°C respectively in incubator for not less than 14 days.

Accelerated Stability studies: Stability studies were carried out on all the prepared ocusert formulas by storing triplicates of each formula (packaged in aluminum foil) in a humidity chamber with a relative humidity of  $75 \pm 5\%$  and a temperature of  $40^\circ \pm 0.5^\circ\text{C}$  [26]. Samples were withdrawn three times with one month time interval between each withdraw, and evaluated physicochemical for their appearance, weight and drug content as described for freshly-prepared ocuserts.[6]

### **M. Accelerated Stability studies**

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### **N. HET CAM Study**

An alternative to the Draize in vivo rabbit eye test for identifying ocular irritations is the HET CAM (Hen's egg chorioallantoic membrane) research. Ten-day fertilized eggs were used for the hen's egg chorioallantoic membrane bioassay. The eggs were candled to determine the embryo's viability before being used. Ocular Solution was compared to a commercial formulation. Sodium hydroxide was used as a positive control and sodium chloride as a negative control in triplicate experiments. After applying 500 µl of the sample to the CAM, the degree of irritation was assessed by looking for symptoms including bleeding, lysis, and coagulation at various intervals up to five minutes. The following formula was used to determine potential

irritation scores (PIS). [7]

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

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.

#### O. Ocular Irritation Studies: In-vivo

**Tolerance Assay:** To ascertain the ocular tolerance of inserts, an acute tolerance test was conducted on male New Zealand white rabbits weighing between 1.64 and 1.76 kg. Every animal was in good health and did not exhibit any clinically noticeable abnormalities. The animals were kept alone in conventional cages in a room with 12-hour light and 12-hour dark cycles, 20–24°C, 30–75% relative humidity, and unrestricted access to food and drink. The CPCSEA Guidelines for the Care and Use of Laboratory Animals were followed while conducting the study. Furthermore, the ARVO (Association of Research in Vision and Ophthalmology) declaration on the use of animals in ophthalmic and vision research states that rabbits are exclusively fed a green diet. The ophthalmic product's propensity for eye irritation led to the development of the modified Draize procedure. The rabbits were then split into three groups at random, with three

rabbits in each group. Three sets of nine male New Zealand rabbits each were created, labeled as test, positive control, and negative control, respectively, and housed in different cages. The test group received the sterile best formulation, the positive control group received the standard marketed formulation (Eye Drop), and the negative control group received 0.9% NaCl. For seven days, two to three drops of the test solution were typically placed in the lower cul-de-sac once a day, and irritation was measured one hour, twenty-four hours, seventy-two hours, and one week after administration. Periodically, the rabbits' eyes were checked for redness, edema, and wetness. Ocular inserts (F3) were selected for in vivo study. The first group (group I) received the normal saline solution the second group (group II) received the drugs ocular inserts belonging to F3, However, the third group (group III) received the Ocular solution (Eye Drop Marketed

Formulation). Each investigated ocular insert formulation (50 $\mu$ l) was placed in the lower cul-de-sac of rabbit's right eye. 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.[7]

### Results and Discussions:

All the prepared ocular films and inserts were evaluated for their physical appearance. The parameters such as transparency, colour, texture, and other defects were studied. The prepared ocuserts were uniform in appearance with smooth texture and no visible cracks or imperfection.[8][9][10]

**Table No.3: Results of Thickness, folding endurance, weight uniformity and drug content uniformity of prepared Ocular Inserts**

| Sr. No. | Formulation | Thickness (mm) (AM $\pm$ SD) | Folding endurance | Weight Uniformity | Drug content uniformity |
|---------|-------------|------------------------------|-------------------|-------------------|-------------------------|
| 1       | F1          | 0.242 $\pm$ 0.0278           | 63 $\pm$ 1.3      | 3.94 $\pm$ 0.7400 | 96.25 $\pm$ 0.05%       |
| 2       | F2          | 0.264 $\pm$ 0.0134           | 66 $\pm$ 0.8      | 3.23 $\pm$ 0.813  | 96.71 $\pm$ 0.03%       |
| 3       | F3          | 0.228 $\pm$ 0.00164          | 68 $\pm$ 1.8      | 3.35 $\pm$ 0.456  | 98.95 $\pm$ 0.03%       |
| 4       | F4          | 0.287 $\pm$ 0.0645           | 71 $\pm$ 0.9      | 3.56 $\pm$ 0.367  | 97.79 $\pm$ 0.06%       |
| 5       | F5          | 0.265 $\pm$ 0.00734          | 64 $\pm$ 0.8      | 3.05 $\pm$ 0.468  | 97.67 $\pm$ 0.04%       |
| 6       | F6          | 0.238 $\pm$ 0.00895          | 62 $\pm$ 1.0      | 3.53 $\pm$ 0.236  | 96.82 $\pm$ 0.03%       |
| 7       | F7          | 0.289 $\pm$ 0.0256           | 61 $\pm$ 1.4      | 3. 89 $\pm$ 0.478 | 97.56 $\pm$ 0.05%       |
| 8       | F8          | 0.276 $\pm$ 0.00934          | 66 $\pm$ 0.98     | 3.67 $\pm$ 0.398  | 96.49 $\pm$ 0.06%       |
| 9       | F9          | 0.284 0.00878                | 60 $\pm$ 0.87     | 3.56 $\pm$ 0.243  | 96.87 0.04%             |

**Table No. 4: Results of Percentage moisture absorption, surface pH, Swelling index of prepared Ocular Inserts.**

| Sr. No. | Formulation | % Moisture Loss  | Surface pH     | SI         |
|---------|-------------|------------------|----------------|------------|
| 1       | F1          | 4.35 $\pm$ 0.467 | 6.6 $\pm$ 0.03 | 13 $\pm$ 5 |
| 2       | F2          | 4.18 $\pm$ 0.634 | 6.6 $\pm$ 0.04 | 18 $\pm$ 3 |
| 3       | F3          | 2.07 $\pm$ 0.141 | 6.5 $\pm$ 0.02 | 12 $\pm$ 5 |

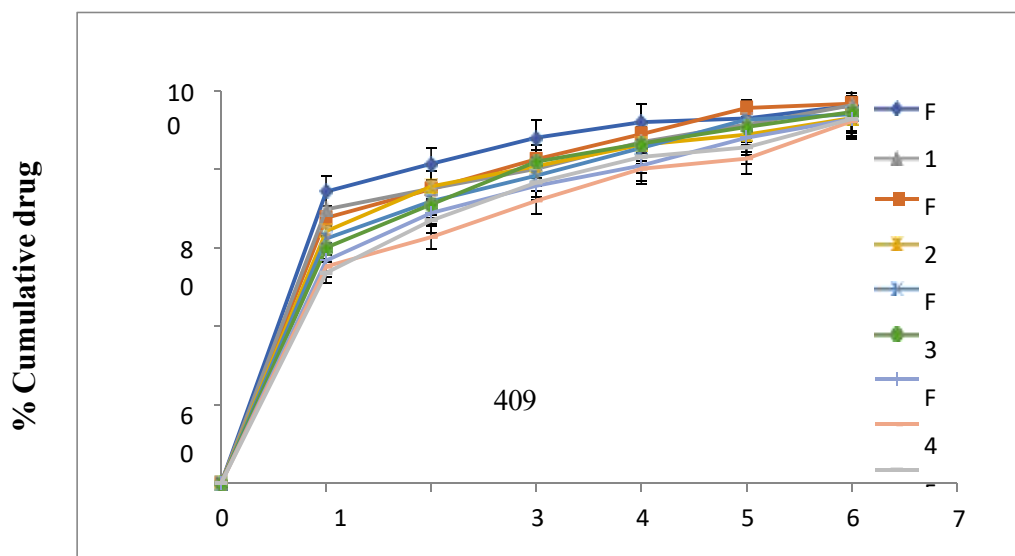
|   |    |                  |                |            |
|---|----|------------------|----------------|------------|
| 4 | F4 | $2.24 \pm 0.418$ | $6.7 \pm 0.05$ | $15 \pm 5$ |
| 5 | F5 | $3.17 \pm 0.367$ | $6.6 \pm 0.04$ | $18 \pm 3$ |
| 6 | F6 | $2.22 \pm 0.544$ | $7.2 \pm 0.03$ | $14 \pm 5$ |
| 7 | F7 | $3.16 \pm 0.236$ | $6.6 \pm 0.04$ | $13 \pm 3$ |
| 8 | F8 | $4.03 \pm 0.321$ | $7.1 \pm 0.04$ | $15 \pm 5$ |
| 9 | F9 | $2.15 \pm 0.255$ | $6.7 \pm 0.06$ | $18 \pm 3$ |

**In vitro drug release study:** The dialysis membrane clamped between the donor and receptor compartments of the Franz diffusion cell was used for the in vitro permeation investigations of Timolol Maleate and Dorzolamide ocular inserts. The outcome shows that medication release reduces as HPMC K4M concentration rises. A release retardant is HPMC K4M. The in vitro drug release from ocular inserts (F1 to F9) was sustained for 6 hours, according to the data displayed in figure. The F3 batch was determined to be more wanted than the other batches.

**Figure No. 1: Franz- Diffusion Cell**



**Figure No. 2: Comparative *in vitro* release profile of Timolol Maleate and Dorzolamide hydrochloride from ocular inserts**



The formulations F1 showed 88.54% and 82.73% release for Timolol maleate and Dorzolamide hydrochloride respectively whereas F2 and F4 showed 80.18% - 93.16% and 91.89% - 94.70% release at the end of 24 hours respectively. The formulations F3 showed 96.86% and 97.91% release at the end of 72 hours respectively whereas F5, F6 showed release of 87.18% - 91.13% and remaining formulation showed in between 90.18% - 92.25% release in 48 hours respectively for Timolol maleate and Dorzolamide hydrochloride. The formulation F3 showed the potential of sustaining the drug release for the longest period of time and hence, it was selected as optimized formulation for further test.[11]

### Effect of formulation variables on drug content:

Statistical Analysis of the central composite design batches was performed by multiple regression analysis using Design of Experiment (version 10) software, results of ANOVA shown below table.

### Response 1: Drug Content

| Source           | Sum of Squares | df | Mean Square | F-value | p-value |             |
|------------------|----------------|----|-------------|---------|---------|-------------|
| <b>Model</b>     | 35.41          | 5  | 7.08        | 191.40  | 0.0006  | significant |
| HPMC K4M         | 6.00           | 1  | 6.00        | 162.00  | 0.0010  |             |
| Propylene Glycol | 0.0000         | 1  | 0.0000      | 0.0000  | 1.0000  |             |
| AB               | 1.0000         | 1  | 1.0000      | 27.00   | 0.0138  |             |
| A <sup>2</sup>   | 14.23          | 1  | 14.21       | 384.00  | 0.0003  |             |
| B <sup>2</sup>   | 14.23          | 1  | 14.22       | 384.00  | 0.0003  |             |
| <b>Residual</b>  | 0.1110         | 3  | 0.0370      |         |         |             |
| <b>Cor Total</b> | 35.55          | 8  |             |         |         |             |

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 191.40 implies the model is significant. There is only a 0.06% chance that an F-value this large could occur due to noise.

**P-values** less than 0.0500 indicate model terms are significant. In this case A, AB, A<sup>2</sup>, B<sup>2</sup> are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

## Fit Statistics

|                  |        |                                |         |
|------------------|--------|--------------------------------|---------|
| <b>Std. Dev.</b> | 0.1925 | <b>R<sup>2</sup></b>           | 0.9969  |
| <b>Mean</b>      | 92.78  | <b>Adjusted R<sup>2</sup></b>  | 0.9917  |
| <b>C.V. %</b>    | 0.2074 | <b>Predicted R<sup>2</sup></b> | 0.9768  |
|                  |        | <b>Adeq Precision</b>          | 43.4871 |

The ANOVA for the dependent variables demonstrates that the model was significant for all response variables and the effect is like, the amount of HPMC K4M and Propylene Glycol 400 were found to be significant, along with its quadratic and interaction terms for all the dependent variables.[12]

**Analysis of Contour plot and response surface model:**

Figure displays 2D contour plots, which are excellent for examining how a component interacts with the responses. These kinds of plots are helpful for examining the simultaneous impact of two variables on the answer and the overall given figure. The effects of X1 (HPMC K4M) and X2 (Propylene Glycol 400) with their non- interaction on drug content at a fixed or level X2 are all linear up to a specified range. The counterplot makes it clear that the formulation benefits from the increased amount of both X1 and X2.

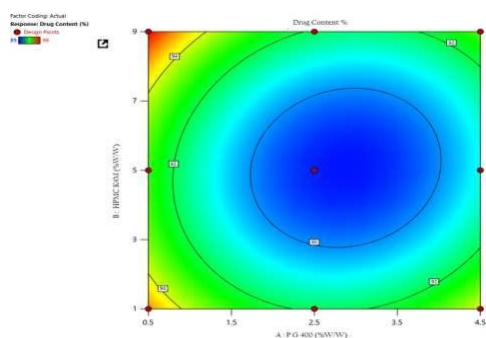
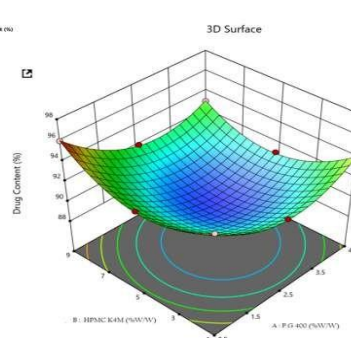
**Figure No3.: Contour plot of drug content****Figure No.4: Response surface plot (3D) of drug content**

Figure shows three-dimensional response surface plots, which are highly helpful for examining how different factors interact to affect responses. These charts are highly helpful for forecasting the outcomes of experiments. These diagrams are highly helpful for understanding the link between the dependent and independent variables, as well as the simultaneous effects of two components.

**Ex-Vivo Tran's 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 ocular insert. Cumulative percent drug permeated from Timolol maleate and Dorzolamide hydrochloride ocular insert (batchF3) was found to be 96.86 % which is shown in Figure

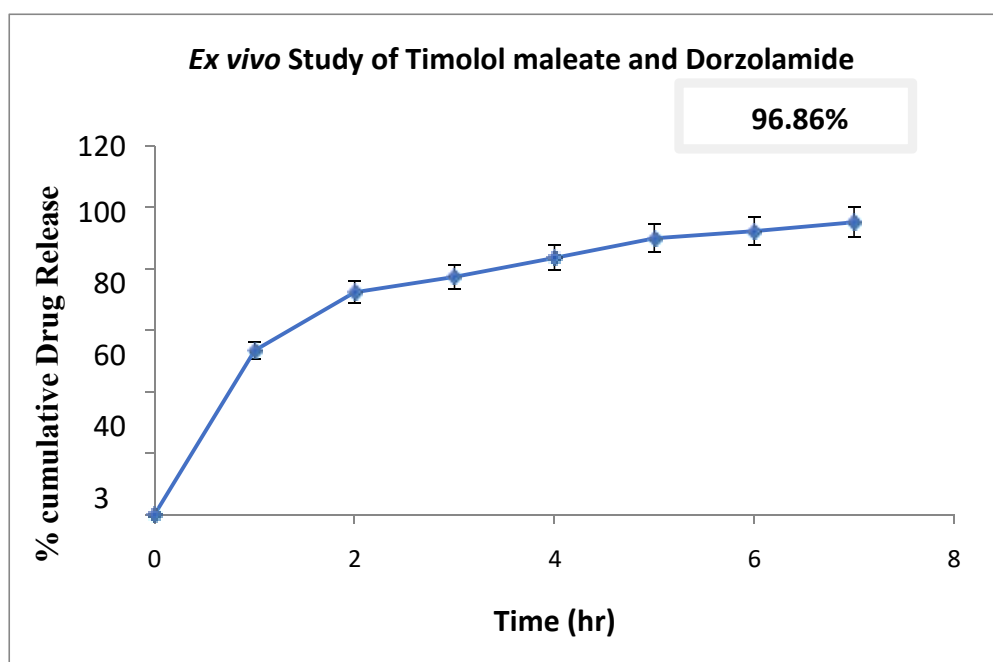


**Figure No. 5: *Ex-vivo* trans corneal cumulative % drug release**

**Table No.5: The regression coefficient of kinetic model**

| Kinetic model            | r <sup>2</sup> |
|--------------------------|----------------|
| Zero-order               | 0.7345         |
| First-order              | 0.9578         |
| Higuchi                  | 0.9312         |
| Korsemeyer-Peppas        | 0.8678         |
| Hixson-Crowell cube root | 0.9998         |

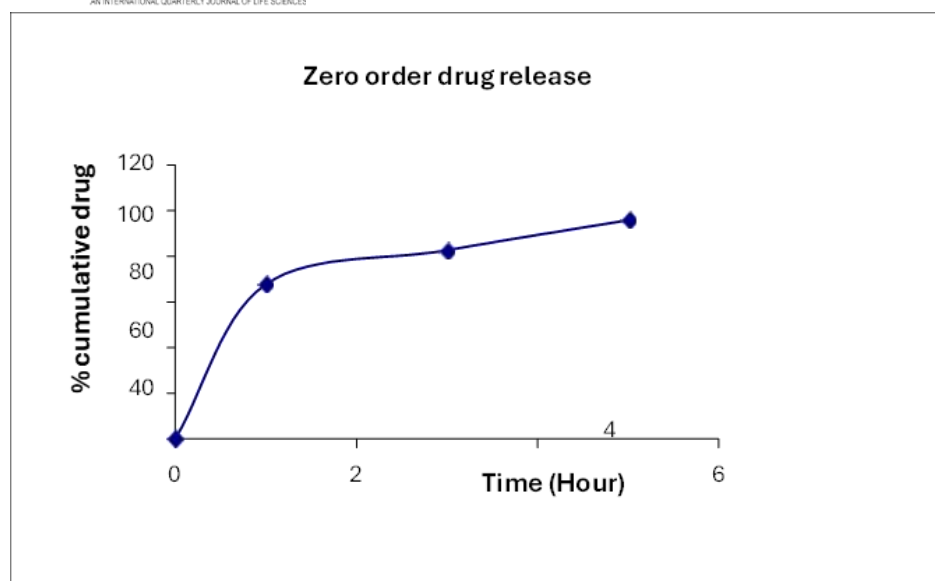
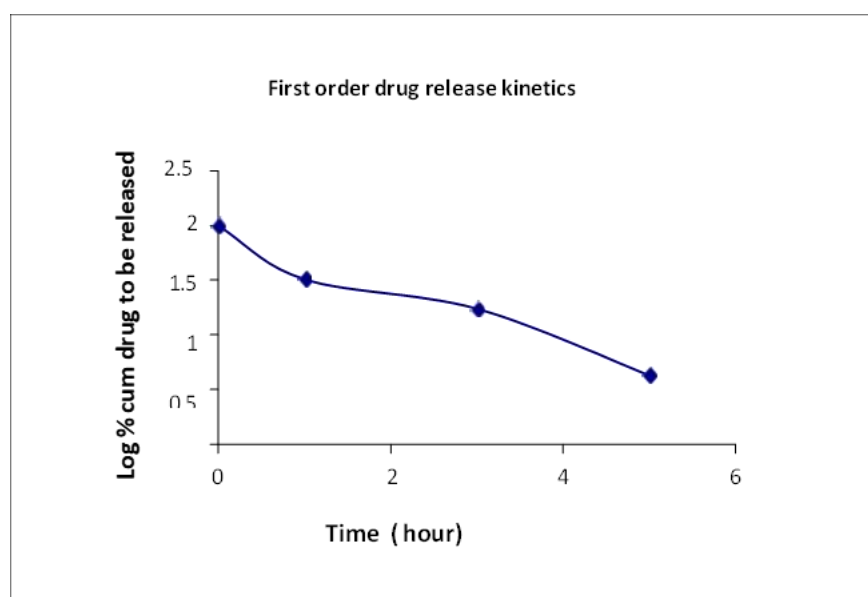
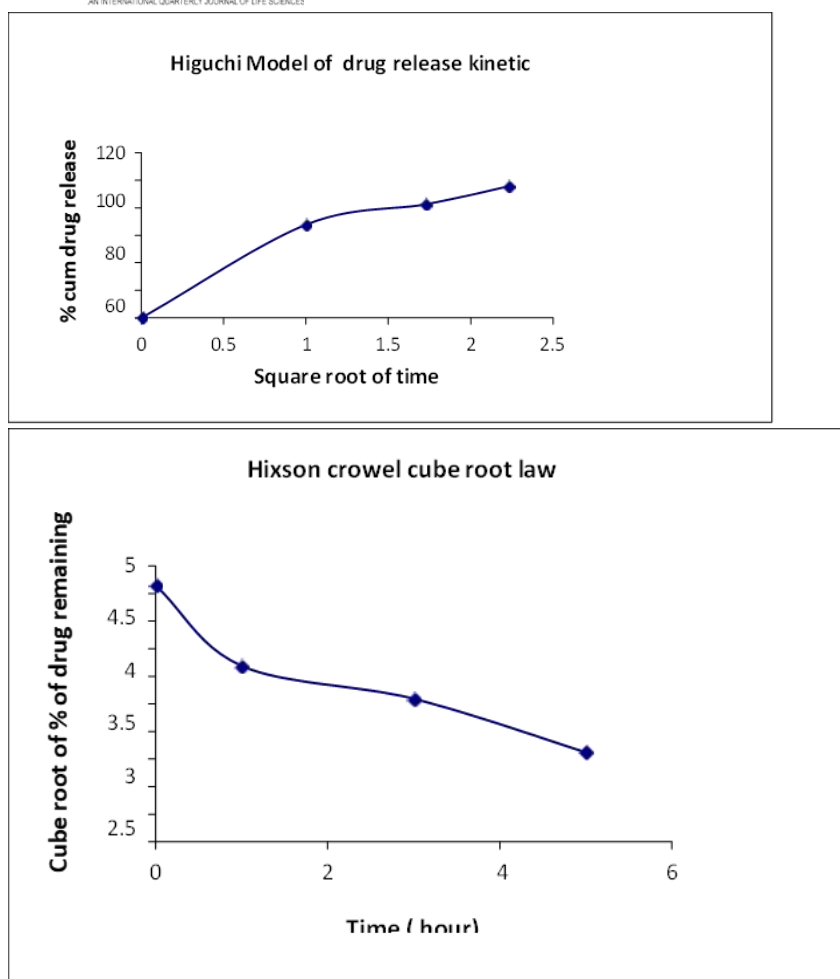
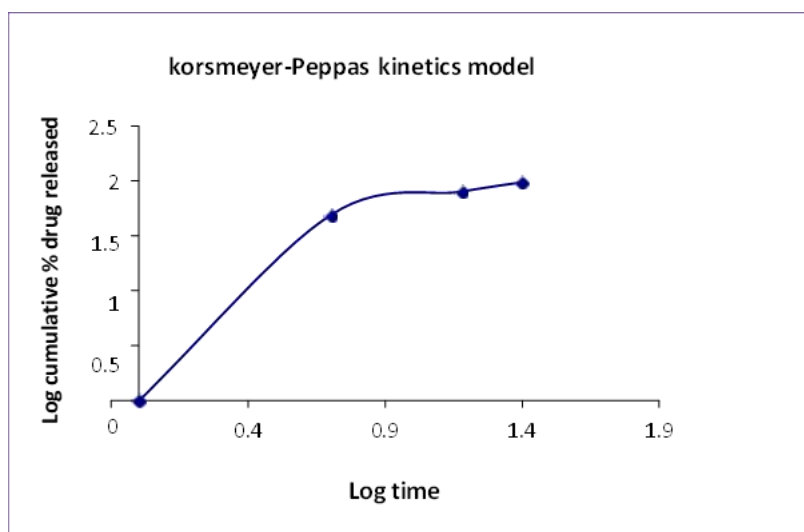


**Figure No.7: First Order drug release Kinetic**



**FigureNo.8: Higuchi Model for Drug Release Kinetic**

**igure No.9: Hixson Crowel Cube Root Law****Figure No.10: Korsmeyer –Peppas drug release kinetics**

**Sterility Testing of ocular inserts as per IP 2014:** After sterilization, the existence of microorganisms is examined in this investigation. Gamma radiation was used to disinfect the ocular inserts. In this investigation, the presence of bacteria was examined using alternative thioglycolate medium (ATGM), while the presence of fungi was examined using soybean case in digest media (SBCD). The media's turbidity is a sign that microorganisms are present. Sterility testing was conducted using optimized batch component films; the findings are displayed in the table and figure.[13][20][23]

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

**Table No.6: Sterility Testing for Batch F3**

In above Table (-) sign indicates no growth of microorganisms and (+) sign indicate the growth of microorganisms. Optimized batch F3 component films were free from microorganism's contamination when formulated in the sterile aseptic conditions

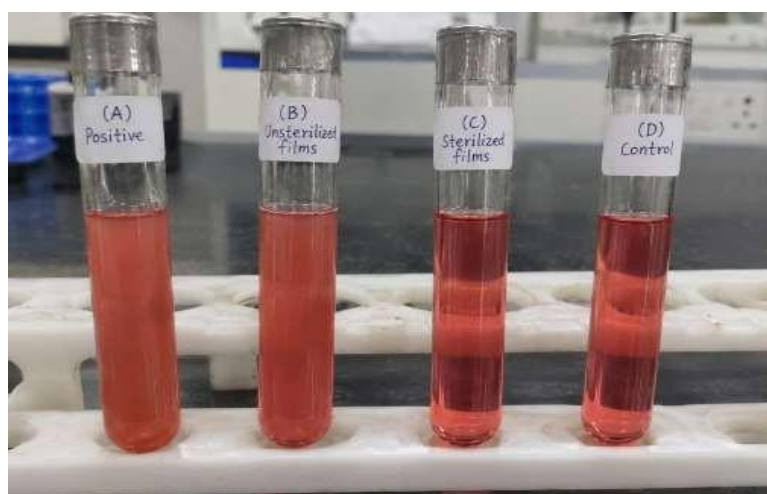


**Figure No. 11: Sterility testing**

**Stability study:** All the selected formulations were subjected to stability testing for the period of three months as per ICH norms at a temperature of  $40\pm 2^{\circ}\text{C}$ ,  $75\pm 5\%$  RH.

**Table No.7: Stability study of Inserts in drug content**

| Sr. No. | Formulation | Drug Content | Drug Release |
|---------|-------------|--------------|--------------|
| 1       | F1          | $89\pm 1.52$ | $87\pm 1.24$ |
| 2       | F2          | $92\pm 1.21$ | $83\pm 1.39$ |
| 3       | F3          | $99\pm 1.89$ | $98\pm 1.69$ |
| 4       | F4          | $94\pm 1.59$ | $91\pm 0.81$ |
| 5       | F5          | $80\pm 1.62$ | $94\pm 1.22$ |
| 6       | F6          | $95\pm 1.58$ | $93\pm 0.91$ |
| 7       | F7          | $96\pm 1.23$ | $92\pm 1.25$ |
| 8       | F8          | $92\pm 1.57$ | $84\pm 1.40$ |
| 9       | F9          | $94\pm 1.89$ | $83\pm 1.59$ |

The stability of selected formula (F3) was studied  $40^{\circ}\text{C}/75\%$  RH for three months. Samples the Ocular insert was taken at one month interval and was studied for drug content. After the stability study, formulation F3 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.[14][21]

**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 (F3). Hence both found to be non-irritant. This study reveals that the D-ocular insert is non-irritant and tolerable shown in table.

**Table No.8: Standard HET-CAM score followed as evaluation parameter for Ocular Tolerance**

| Sr. No. | Effect of Formulation on CAM                                            | Score | Presumption       |
|---------|-------------------------------------------------------------------------|-------|-------------------|
| 1       | No visible hemorrhage                                                   | 0     | No irritation     |
| 2       | Observable membrane discoloration                                       | 1     | Mild- Irritant    |
| 3       | Structure cover partially the area of CAM due to membrane discoloration | 2     | Moderate Irritant |
| 4       | Structure fully covers the 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.[15][22]

Initial mild redness and lacrimation that lasted for less than 1 min was observed in rabbit's eye (group II) after direct instillation of Timolol Maleate and Dorzolamide Hydrochloride 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 Timolol Maleate and Dorzolamide Hydrochloride ocular inserts (F3) 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.



**Figure .12 In-vivo Tolerance Study**

**Table no.9: grading for Rabbit Eye irritation Study[16][17]**

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

**Table no.10: *In-Vivo* Tolerance Study[17][18][19]**

| <b><i>In-Vivo</i> Tolerance Study</b> |                                                  |                  |                 |               |               |
|---------------------------------------|--------------------------------------------------|------------------|-----------------|---------------|---------------|
| <b>Groups</b>                         | <b>Treatment</b>                                 | <b>Parameter</b> | <b>0.5 Hrs.</b> | <b>1 Hrs.</b> | <b>2 Hrs.</b> |
| <b>Gr. 01</b>                         | Normal Saline Solution (3 Animal)                | Redness          | 1.344 ± 0.566   | 0.344 ±0.566  | 0             |
|                                       |                                                  | Inflammation     | 0.344 ±0.566    | 0             | 0             |
|                                       |                                                  | Tear Production  | 1.655 ±0.566    | 0             | 0             |
| <b>Gr. 02</b>                         | Timolol and Dorzolamide Ocular Insert (3 Animal) | Redness          | 2 ±1            | 0.344 ±0.566  | 0             |
|                                       |                                                  | Inflammation     | 0               | 0             | 0             |
|                                       |                                                  | Tear Production  | 1 ±0            | 0.344 ±0.566  | 0             |
| <b>Gr. 03</b>                         | Timolol and Dorzolamide Eye Drop (3 Animal)      | Redness          | 2.655 ±0.566    | 2.344 ±0.566  | 0             |
|                                       |                                                  | Inflammation     | 0               | 0             | 0             |
|                                       |                                                  | Tear Production  | 2.655 ±0.566    | 2.655 ±0.566  | 0             |

## Conclusion:

The study successfully achieved its objective of developing a stable, flexible and sustained-release ocular insert containing Timolol Maleate and Dorzolamide Hydrochloride. The optimized formulation, F3, exhibited satisfactory physicochemical properties, drug content uniformity, controlled drug release and stability. The developed RP-HPLC method provided a reliable analytical tool for estimation of both drugs. The findings of the study indicate that the developed ocular insert system has potential as an alternative sustained-release ophthalmic drug-delivery approach, capable of maintaining drug release over an extended period and potentially reducing the frequency of conventional ocular drug administration. However, further investigations such as ex-vivo corneal permeation, ocular irritation studies, and pharmacokinetic evaluation and in-vivo efficacy studies translation of the optimized formulation toward clinical or commercial application.

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