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Ocular insert of Timolol Maleate using naturally occurring biodegradable polymer

Revathy V. Nair, Sreeja C. Nair and Anoop K. R.*

Department of Pharmaceutics, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham University, AIMS Health Sciences Campus, Kochi, India

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ABSTRACT

Conventional ocular preparation like eye drop, solutions or suspensions often result in poor bioavailability and patient compliance. Timolol maleate is a beta adrenoceptor blocker widely used in treatment of glaucoma in the form of eye drop. The study aimed in preparing Timolol Maleate ocular inserts (ocuserts) enhancing ocular bioavailability and the reduction in the frequency of instillation thereby resulting in better patient compliance. Timolol maleate ocuserts were prepared by using solvent casting method using a hydrophilic polymer (Sodium alginate) and polyethylene glycol as a plasticizer. Six different ocuserts (F1-F6) were evaluated for pH, weight variation, thickness, folding endurance, percentage drug content, moisture absorption, moisture loss, in-vitro drug release by using dialysis membrane. In-vitro drug release data of optimized formulation (F4) was treated according to Zero, First, Korsmeyer Peppas and Higuchi kinetics to access the mechanism of drug release. Its sterility test was performed based on IP guidelines. From the parameters, the ideal formulation was identified as F4 with pH (7.4), weight variation(5.50 mg), thickness(0.085 mm), folding endurance (89), percentage drug content (97.27%), moisture absorption(4.17%), moisture loss(3.42%), in-vitro drug release (95% at 10 hours). F4 showed first order release pattern. The sterility test of ocusert F4 was performed by using alternate thioglycollate medium and soyabean casein digest medium, which confirmed that the ocusert F4 passed the sterility test and hence they are sterile preparations. Thus the developed optimized ocusert with its sustained release property can be utilized as an alternative to conventional dosage form for the treatment of Glaucoma.

Keywords: Hydrophilic polymers, Dialysis membrane, Sterility test, Ocular insert.

INTRODUCTION

Ophthalmic drug delivery is one of the most interesting and challenging endeavors faced by the pharmaceutical scientist. The complex anatomy, physiology and biochemistry of the eye render this organ exquisitely impervious to foreign substances. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage. Conventionally ophthalmic preparations are in the form of solutions, suspensions and ointment dosage forms are clearly no longer sufficient to combat some present virulent diseases[1] .Due to tear drainage, most of the administered dose passes via the naso-lacrimal duct into the GI tract, leading to side effects. Rapid elimination of the eye drops administered often results in a short duration of the therapeutic effect making a frequent dosing regimen necessary. Ocular therapy would be significantly improved if the precorneal residence time of drugs could be increased[2].Timolol maleate is a beta adrenoceptor blocker widely used in treatment of glaucoma[3] in treatment of open angled glaucoma Timolol maleate is given in divided dose several time in a day in the form of eye drop[4].Sodium alginate is a natural polysaccharide extracted from marine brown algae, is a hydrophilic polymer which is mucoadhesive, biodegradable, and biocompatible in nature and has potential for numerous pharmaceutical and biomedical applications such as drug delivery system and cell encapsulation[5] .In this study, an attempt was made to prepare ocular insert with the basic objective of increase pre corneal residence time, reducing the frequency of administrations and thus enhance the patient compliance and therapeutic efficacy.

EXPERIMENTAL SECTION

Timolol maleate was received as a gift sample from FDC Pharma Pvt. Ltd, Aurangabad. Sodium alginate and polyethylene glycol was purchased from Nice chemicals, Kochi. Dialysis membrane was procured from Himedia. Other chemicals and solvents used in the study were of analytical grade.

A. Preliminary Studies:

1. Determination of lambda max of drug:

An absorption maximum of Timolol maleate was determined using phosphate buffer (pH 7.4). Solutions ranging from 20-30µg/ml were scanned from 200-400 nm using spectrophotometer.

Estimation of Timolol Maleate:

100 mg of Timolol Maleate was accurately weighed and was dissolved in 100 ml of simulated tear fluid (STF pH 7.4) to generate a stock solution having concentration of 1mg/ml. Stock solution (10 ml) was further diluted to 100 ml to produce standard solution having concentration of 100µg/ml. The standard solution was serially diluted with STF pH 7.4 to get working standard solutions having concentration of 2, 4, 6, 8, 10, 12, 14, 16,18 and 20 µg/ml. The absorbance of the solutions was measured at 294nm using double beam UV visible spectrophotometer against STF pH 7.4 as a blank. The plot of absorbance v/s concentration (µg/ml) was plotted (Figure5.1) and data was subjected to linear regression analysis in Microsoft Excel[8].

Preparation of Simulated Tear fluids (STF) pH 7.4:

Table 1: Composition of Simulated Tear fluids

INGREDIENTS	QUANTITY
Sodium chloride	0.67 g
Sodium bicarbonate	0.20 g
Calcium chloride	0.08 g
Distilled water	100 ml

2. Solubility analysis [9]

Solubility of Timolol Maleate was determined in different solvents.

3. FTIR[10]

The FTIR spectra of the pure drug (Timolol maleate, Polymer (sodium alginate) and Ocusert (Timolol Maleate, Sodium Alginate and PEG) were taken as KBr pellets in the range of 4000–650 cm^{-1} (FT/IR-4100 type A spectrophotometer, Jasco, Japan). The infrared analysis of optimized insert was carried out in the same range by ATR-IR spectroscopy (Perkin Elmer Model 1600 FT-IR spectrophotometer with ATR mode Perkin Elmer,USA).

4. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) scans of pure drug and drug loaded ocular insert were performed using DSC 1/700 (Mettler Toledo, Germany).The analysis was performed with a heating range of -25 ^{o} C to 250 °C and at a rate of 10 °C/min in nitrogen atmosphere. The sample weight was approximately 6 mg.

Table 2: Composition of different ocular inserts

FORMULATION	DRUG CONCENTRATION (mg)	SODIUM ALGINATE CONCENTRATION (mg)	AMOUNT OF PEG (ml)	DISTILLED WATER (ml)
F1	0.5	0.05	2	10
F2	0.5	0.10	2	10
F3	0.5	0.15	2	10
F4	0.5	0.20	2	10
F5	0.5	0.25	2	10
F6	0.5	0.30	2	10

Formulation Method[6,7]

The ocular inserts were prepared by solvent casting method. Weighed quantity of polymer Sodium Alginate was dissolved in 10 ml distilled water under continuous stirring as per the quantity mentioned in Table.2. Timolol maleate equivalent to 0.450 mg per ocular insert was added to the polymeric solution. The medicated polymer solution was sonicated for half an hour to remove air bubbles. Then plasticizer PEG- 400 30% of dry weight of polymer was added under continuous stirring. Then resultant solution was kept aside to get uniform distribution for ten minutes. After proper mixing the casting solution was poured on a petri plate (Diameter 6.5 cm and area 20.41 cm^2) and covered with inverted funnel to allow slow and uniform evaporation of solvent for 24 h. The dried film

thus obtained was cut by 6 mm cork borer to get ocular insert. Insert was sterilized under UV for 1 min and individual insert was packed in sterilized aluminum foil which was further stored in desiccator at room temperature.

B. Physicochemical evaluation/characterization of ocular inserts:
1. Physical appearance
All the ocular inserts were visually observed for color, clarity and smoothness of its surface.

2. Surface pH [11]
Surface pH of the ocular inserts was determined by allowing them to swell in a closed petri dish at room temperature for 30 min in 0.1 mL of distilled water. The tip of pH meter was gently placed over the swollen devices and the surface pH was determined.

3. Thickness[11]
The thickness of the formulated inserts was measured using digital micro meter of sensitivity of 0.01mm. Average of 3 readings was taken and standard deviation values were calculated.

4. Weight uniformity[12]
Evaluation was carried out by weighing the inserts by an electronic balance (least count – 0.1 mg). The average weight and standard deviation were then calculated and reported.

5. Folding endurance[13]
Folding endurance was determined by repeatedly folding a small strip of ocular insert (2×2 cm) at the same place till it breaks. The number of times film could be folded at the same place, without breaking gives the value of folding endurance which was recorded.

6. Percentage moisture content[14]
Ocular insert were weighed individually and placed in a desiccator .After three days, inserts were taken out and reweighed. The percentage moisture loss was calculated by using following formula.

$$\text{Percentage moisture content} = \frac{\text{Final weight} - \text{Initial Weight}}{\text{Initial weight}} \times 100$$

7. Determination of drug content[15]
Ocular insert was dissolved in simulated tear fluid pH 7.4. The resultant mixture was transferred to 50 ml volumetric flask and allowed to shake for 1 h. Then after, diluted up to the mark with simulated tear fluid. Similarly blank was prepared using drug free insert. Drug content was determined by UV Spectrophotometer at 294 nm.

8. Surface morphology[16]
Surface characteristics of the polymeric blend were studied by Scanning Electron Microscopy. Inserts were mounted on an aluminum stub using double-sided adhesive carbon tape and coated with gold palladium using JEOL JFC 1600 auto fine coater for 90 sec. Samples were examined using scanning electron microscope JSM-6380 LV (Jeol Ltd., Tokyo, Japan) at 20 kv accelerating voltage.

C. *In vitro* drug release study[17]
For *in vitro* studies of ocular inserts, we used a cylindrical tube which has the diameter of 15 mm. Dialysis membrane overnight soaked in water for followed by rinsing in phosphate buffered saline (PBS) solution, acted as corneal epithelium, was tied to one end of open cylinder which acted as donor compartment. An ocular insert was placed inside this compartment with simulated tear fluid (STF pH 7.4).Then, the glass tube was suspended in the dissolution flask of a USP dissolution apparatus such that entire surface of the membrane was in contact with the receptor compartment containing 250 mL of STF (pH 7.4). The content of the receptor compartment was stirred continuously at 25 rpm. 1 ml samples were withdrawn from the receptor compartment at periodic intervals and replaced by equal volume of fresh solution. The samples were analyzed spectrophotometrically at 294 nm against reference standard using STF as blank.

D. Mechanism of release
The release rate obtained is tabulated and graphed according to the following modes of data treatment:
a) Cumulative percentage drug released Vs time (*in-vitro* diffusion plots)
b) Log percentage drug remained Vs time (First order rate plots)
c) Cumulative percentage drug released Vs Square root of time (Higuchi's plots)
d) Log percentage drug released Vs Log time (Peppa's exponential plots)

E. Sterility testing[18]
Sterility is one of the most vital requirements for an ophthalmic preparation. The tests for sterility are intended for detecting the presence of viable forms of microorganisms in ophthalmic preparations. In the present study, two media namely, Fluid thioglycolate medium and Soyabean-casein digest medium (SBCD) were used[19] .The optimized sterilised inserts were inoculated into the above medium aseptically. The medium was stored to detect the presence of growth of microorganism for next one week.

F. Accelerated Stability Studies[19]
The optimized formulations were stored at 30±2^{0}C/65±5% RH and 40±2^{0}C/75±5% RH for 3 months in stability chamber (Remi, India). The samples were withdrawn at every 1 week time intervals and analyzed for physical parameters and drug content.

RESULTS AND DISCUSSION

In the present study Timolol Maleate loaded ocular insert were prepared using polymer Sodium alginate and PEG as a plasticizer by solvent casting method. The prepared ocusert were evaluated for their use as ocular delivery system with a view to obtain sustained release.

A. Preliminary screening
1. Determination of lambda max of Timolol maleate:

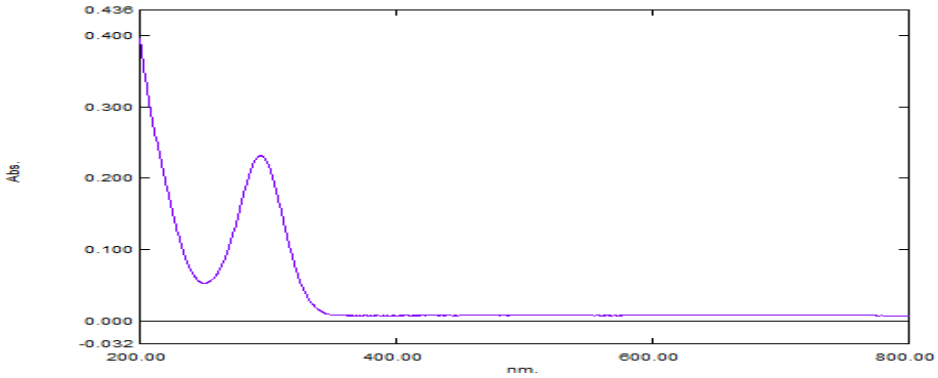

Figure 1: Lambda max of Timolol Maleate

2. Estimation analysis of Timolol maleate

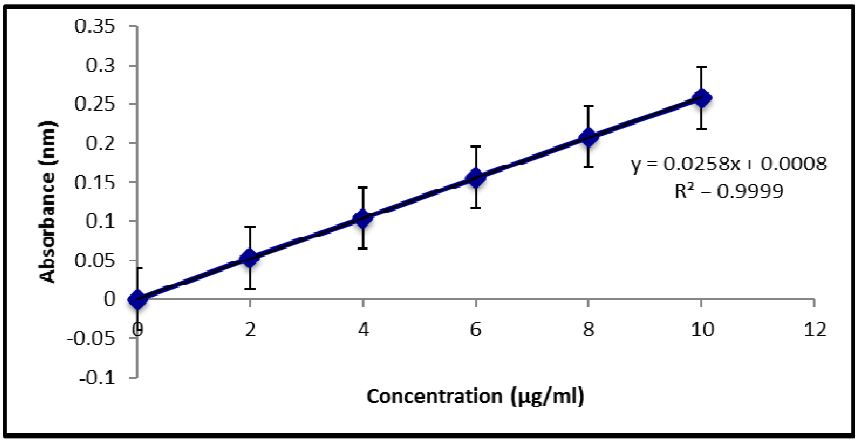

Figure 2: Standard graph of Timolol Maleate in phosphate buffer pH 7.4

2) Solubility analysis:
Solubility of Timolol Maleate was determined in different solvents and the observations are shown in Table 3.

Table 3: Solubility profile of Timolol Maleate in different solvents

Serial no.	Solvents	Solubility
1	Methylene chloride	Soluble
2	0.1N HCl	
3	Chloroform	Freely soluble
4	Methanol	
5	Ethanol	
6	Distilled water	
7	0.1N NaOH	Slightly soluble
8	Ethyl actate	Partialy insoluble

3) FT-IR:

IR spectra analytical reports indicated that there was no interaction between drug and the polymer used.In the spectrum of alginate, the bands around 1022 cm-1 (C-O-C stretching) are attributed to its saccharide structure. In the addition of the bands at 1593, 1402 cm-1 are assigned to asymmetric and symmetric stretching peaks of carboxylate salt groups.[20] It was observed from the spectra of Timolol maleate, Sodium Alginate and mixture of these two confirms that important peaks secondary amide and quaternary amine (3305 cm^{-1}), OH stretching(3295 cm-1), CH stretching aliphatic(2850 cm-1, 2889 cm-1), C=O stretching (1705.73cm-1), C=C aromatic ring (1500 cm-1), C-O-C stretching (1120 cm-1, 1062 cm-1) are present in FTIR spectra of Timolol maleate and mixture of Timolol maleate and Sodium Alginate. It proved that drug and polymer are compatible to each other; there was no interaction between drug and excipients.

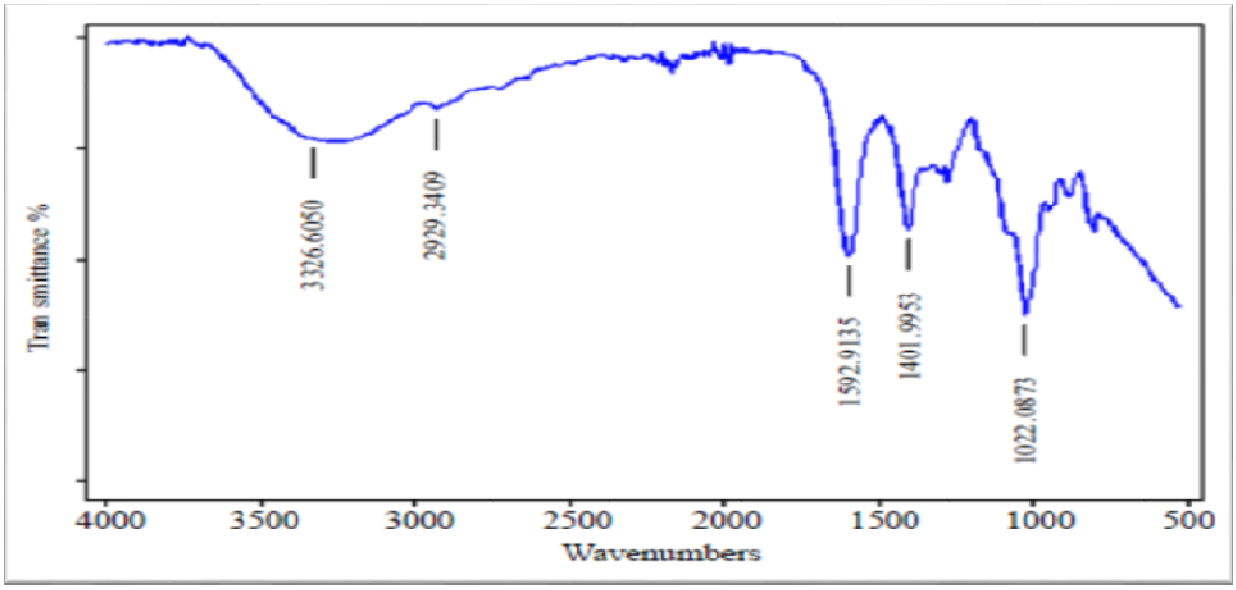

Figure 3: FTIR spectrum of Sodium alginate

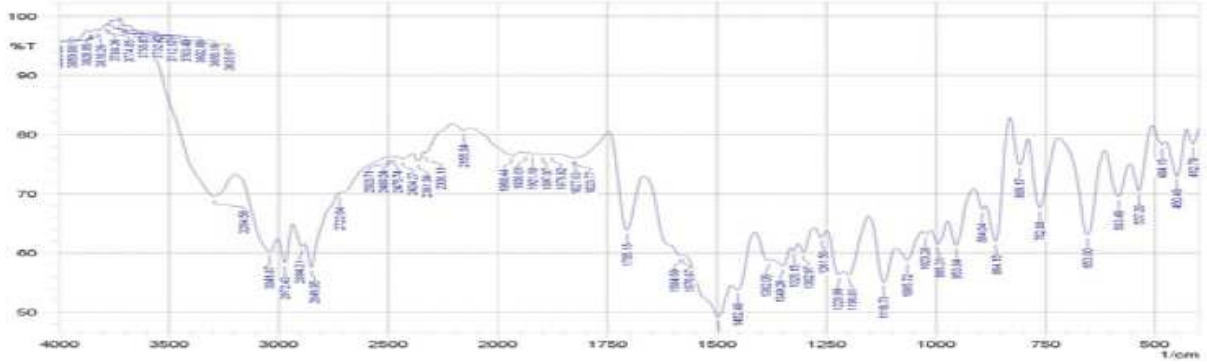

Figure 4: FTIR spectrum of Timolol Maleate

4) DSC:

Thermogram exhibited a sharp melting endotherm at an onset temperature of 112.82^{o}C, a peak temperature of 119.76 oC and a heat of fusion of 6.97 J/g. While the thermogram of film shows crystallization of Timolol Maleate

from glass at 67.18 oC followed by fusion at 116.89^{o} .The thermal behavior of the insert suggested that the drug is present in the insert as semicrystalline form as the fusion peak in the film is very weak compared to the pure drug.

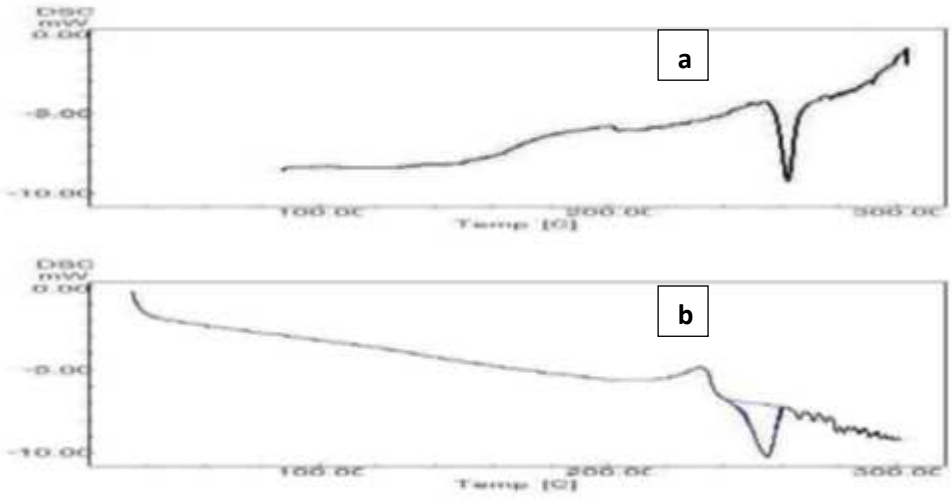

Figure 5: Differential Scanning Calorimetry (DSC) thermogram of (a) Timolol Maleate drug (b) Drug and sodium alginate

Formulation

Six different ocular inserts were prepared by solvent casting method. Sodium alginate being hydrophilic in nature, is easily dissolved in water to which drug was added with continuous stirring to get a uniform distribution of drug within the polymer. PEG was used as a plasticizer in required quantity to obtain an ocular insert having flexible nature.

B. Physico-chemical studies:

All prepared ocular inserts had a good appearance with smooth, semitransparent, uniform surface.

The surface pH of the prepared inserts varied between 6.8 to 7.4 (Table 4) which is comparable with the pH of tear fluid i.e. 7.4. This indicates that the formulations will not produce any irritation in presence of tear fluid when placed inside the cul-de-sac.

The thickness of the prepared insert varies from 0.045 to 0.11 mm (Table 4). Weight and thickness measurements of inserts showed a low standard deviation values ensuring the uniformity of weight and thickness in each film. A good weight uniformity of all formulation indicates an even distribution of drug and the polymers in the polymeric matrix. It was also accounted that weight and thickness of films were increasing with increasing polymer concentration. Formulations were not thick enough to produce any irritation in *cul-de-sac*.

The folding endurance of all the formulations was found to be good, it revealed that the folding endurance decreases with increase in polymer concentration. All the batches exhibited good folding endurance value which withstands external stress.

Moisture content values of inserts were found in range of 1.50 ± 0.07 to 7.05 ± 0.17 %. It also shows that moisture content of inserts increase with increasing amount of sodium alginate due to hydrophilic nature of the polymer.

The drug content of all the six formulations were found to be in the range of 94.89 to98.78 %. (Table 4).Lower values standard deviation revealed uniform distribution of drug which in turn confirms the suitability of the process used in formulation of ocular insert.

Table 4: Physicochemical properties of different formulation

FORMUALTION	PHYSICAL APPEARENCE	SURFACE pH	WEIGHT VARIATION* (mg)	THICKNESS* (mm)	FOLDING ENDURANCE*	DRUG CONTENT* (%)	MOISTURE CONTENT* (%)
F1	Good appearance with smooth surface	Between 6.8-7.4	3.25 ±0.516	0.045 ±0.001	94±11	94.13±1.51	1.45±0.05
F2			4.10 ±0.527	0.061 ±0.002	92±13	95.24±2.03	2.50±0.07
F3			5.01 ±0.516	0.069 ±0.001	90±15	95.52±1.21	3.85 ±0.09
F4			5.50 ±0.316	0.085 ±0.005	89±13	97.27±0.61	4.17 ±0.12
F5			6.12±0.483	0.096 ±0.004	81±14	97.73±0.34	5.47±0.15
F6			7.03 ±0.527	0.11 ±0.006	76±12	98.63±0.32	7.71±0.11

*Mean±SD(*n=3)*

The data obtained for *in- vitro* study were tabulated (Table 5) and represented graphically. It showed controlled pattern of release from all formulations at the end of 10 hours (Fig.-6), in comparison to a marketed eye drop solution. Fast release of drug from formulation F1 occurred may be due to low concentration and hydrophilic nature of polymer. The overall result revealed that as the concentration of polymer increases there was slow release of drug from formulation occurred. From the *invitro* data formulation F4 was selected as the optimized formulation.

Table 5: *Invitro* drug release of different formulation

TIME (hours)	Cumulative percentage drug released					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	14	10	18	27	18	21
2	19	15	28	34	25	28
3	25	22	32	42	28	36
4	29	25	37	53	35	45
5	37	32	41	61	46	52
6	41	37	49	69	53	63
7	52	43	57	74	61	71
8	59	45	63	79	69	77
9	68	55	74	86	80	83
10	72	65	82	95	92	90

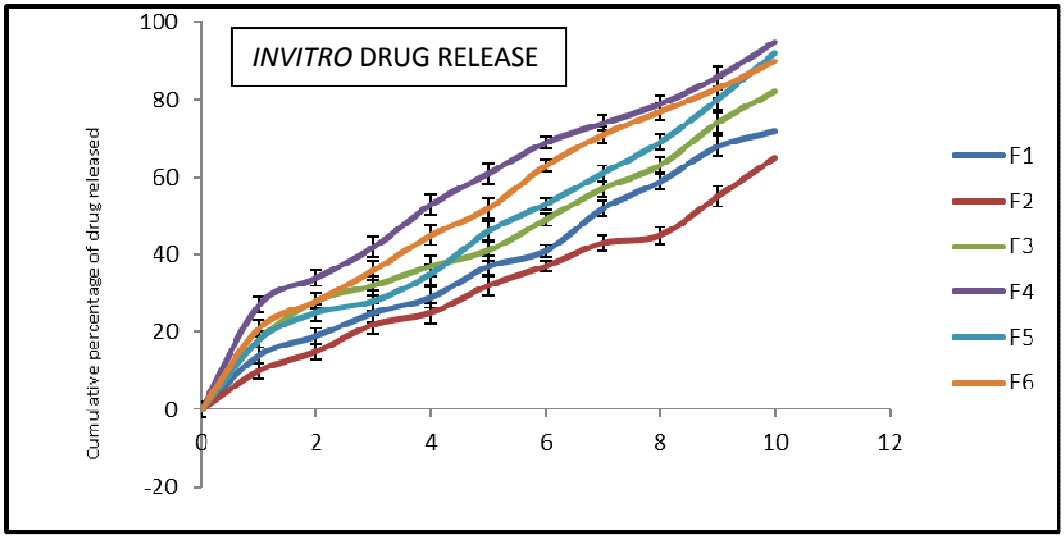

Figure 6: *Invitro* drug release of different ocular formulation

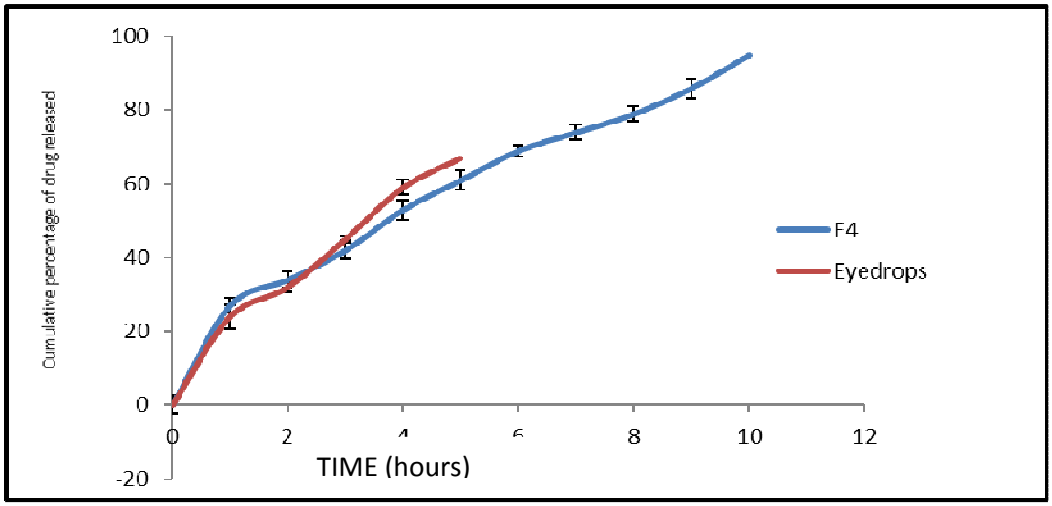

Figure 7: *Invitro* release profile of marketed Timolol maleate eye drop and ocusert

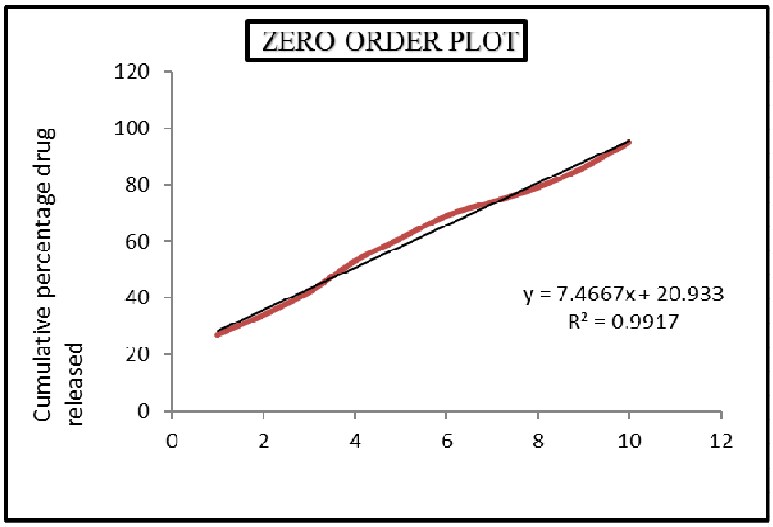

Figure 8: Zero order release profile of timolol maleate ocusert

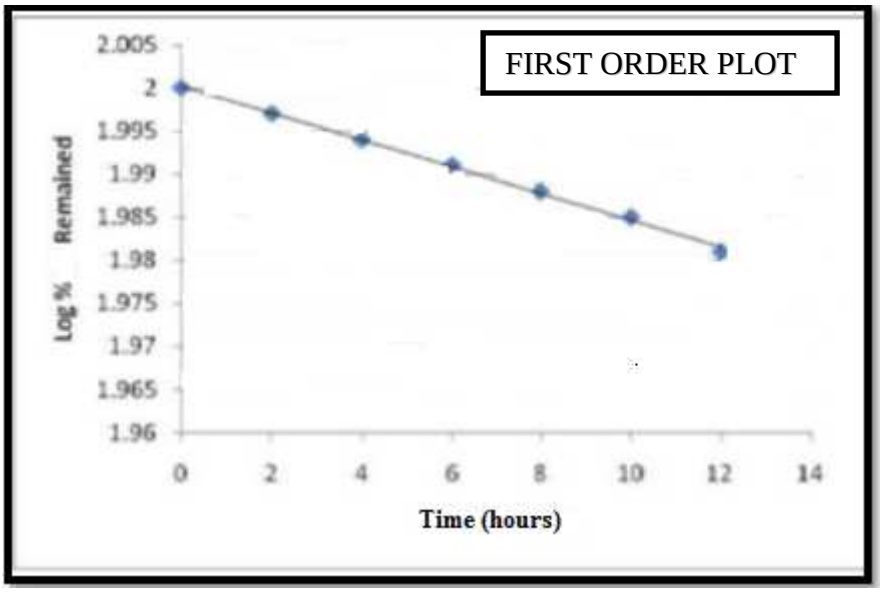

Figure 9: First order release profile of timolol maleate ocusert

Diffusion data were treated with zero order, first order, Higuchi and Kors-Meyer Peppas equation. From the n value it can be seen that all the formulations follow non-fickian diffusion of drug. This can be supported by the good fit of Higuchi equation. The drug was released by diffusion from the polymer matrix. Results also indicated that inserts show zero order drug release at high amount of polymer.

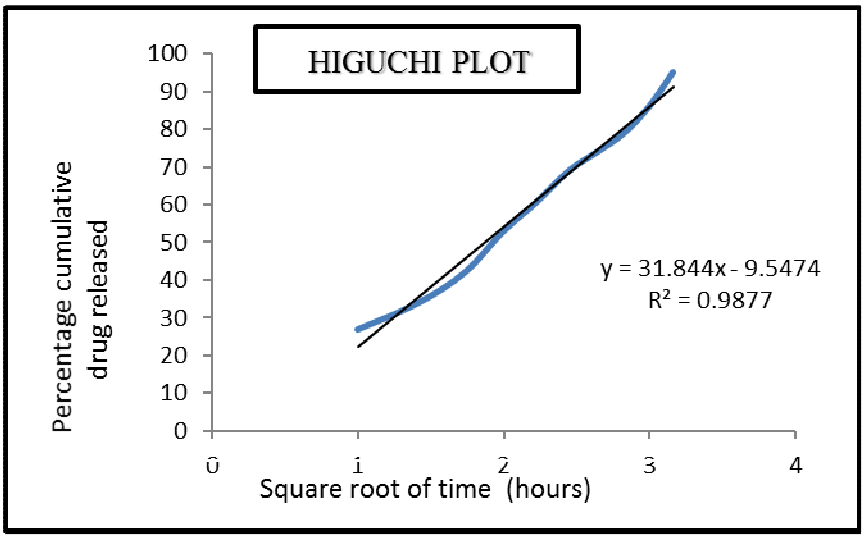

Figure 10: Higuchi plot of Timolol maleate ocusert

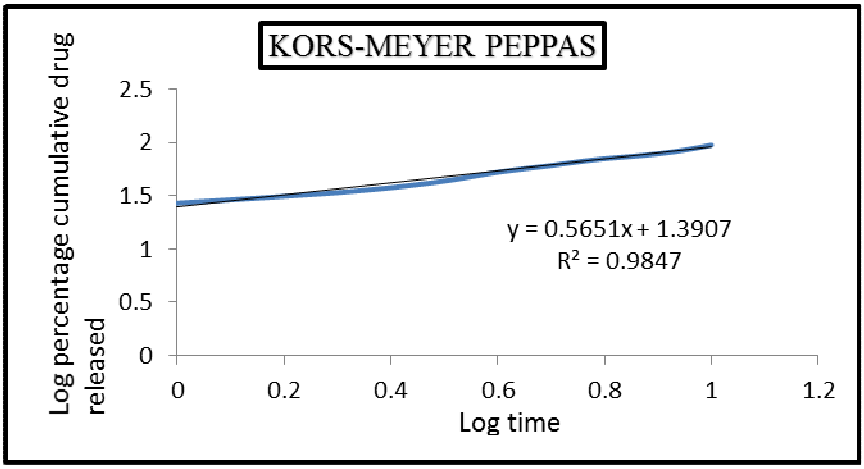

Figure 11: Korsmeyer Peppas plot of timolol maleate ocusert

SEM analysis of F4 formulation (Figure 12) revealed that surface of the ocular insert are smooth indicating the uniform dispersion of drug Timolol maleate within the polymer sodium alginate.

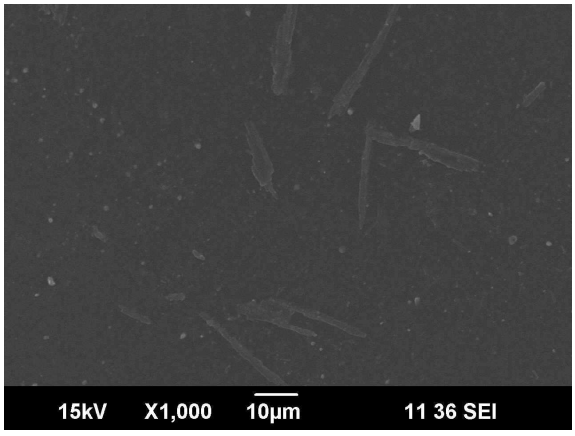

Figure 12: SEM imaging of timolol maleate ocusert

Sterility test was performed for aerobic, anaerobic bacteria and fungi by using fluid thioglycollate medium and soyabean casein digest medium as per the IP'07 procedure. Here, *Bacillus subtilis* was used as a test organism for aerobic bacteria, *Bacterioides vulgatus* was used as test organism for anaerobic bacteria and *Candida albicans* was used as test organisms to detect the presence of fungi. Absence of microbial growth was revealed by Sterility test confirmed that the optimized insert is sterile for ophthalmic purpose.

Table 6: Stability studies of the optimized formulation

TIME PERIOD	PHYSICAL APPEARENCE		pH		DRUG CONTENT(%)	
	40^0C	RT	40^0C	RT	40^0C	RT
Initial	Smooth, transparent surface		6.8-7.4		97.27±0.06	97.27±0.06
After 1 week					97.22±0.12	96.11±0.22
After 2 week					95.25±0.11	95.11±0.23
After 3 week					92.11±0.24	94.21±0.11
After 4 week					87.27±0.66	94.01±0.01

From the results of accelerated stability studies it was found that the formulations were stable and the drug content was found to be within limits at a temperature range of 40 ± 2^0C/75±5% RH.

CONCLUSION

The formulation of Timolol maleate loaded ocusert seems to be promising and further addition of rate controlling membrane may provide controlled release pattern and same fact can be considered for further research. Further *in vivo* study must be carried out to check the therapeutic efficacy of the preparations.

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