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FORMULATION AND IN VITRO - IN VIVO EVALUATIONS OF TIMOLOL MALEATE VISCOUS EYE DROPS FOR THE TREATMENT OF GLAUCOMA

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ABSTRACT
The purpose of this research was to provide comfort and compliance to the patient and yet improve the therapeutic performance of the drug over conventional systems by preparing of functional and patient friendly viscous eye drops of Timolol Malate (TM) for the treatment of glaucoma. Hydroxy propyl methyl cellulose (HPMC K4M) and Carbopol 940 (CP 940) were explored as potential viscosity enhancing vehicles for prolonging the therapeutic effectiveness. The developed viscous eye drops were evaluated for clarity, pH, osmolarity, viscosity, mucoadhasion test and *in vitro* drug release. The optimized formula was subjected for *in vivo* testing by measurement of IOP (intraocular pressure), ocular irritation test and stability studies. TM viscous eye drops containing 1 % HPMC K4M and 0.3 % CP 940 exhibited non-newtonian flow behavior. HPMC K4M and CP 940 were responsible for higher viscosity and mucoadhasion respectively. Developed viscous eye drops exhibited the diffusion mechanism of drug release. The values of slope (n) were in the range of 0.65 to 0.99. IOP measurement of rabbit eye treated with optimized batch showed significant reduction in ocular pressure for 8 hrs. Ocular irritation test in rabbits showed no redness, swelling or watering. The overall study reveals that ratio of HPMC K4M (0.75 mg) and CP 940(0.075 mg) was gave favorable clinical response and used to prolong the therapeutic effectiveness of TM.

Key Words: Carbopol 940, HPMC K4M, Timolol Maleate, Viscous eye drops.

1. INTRODUCTION
Eye is the most unique organ of the body.[1] It is one of the challenging organs for drug delivery because its unique anatomy restricts drug absorption into the deep tissues.[2] A major problem being faced in ocular therapeutics is the attainment of an optimal concentration at the site of action. Poor bioavailability of drugs from ocular dosage forms is mainly due to the tear production, nonproductive absorption, transient residence time, impermeability of corneal epithelium, blinking, reflex lachrymation and drainage remove rapidly foreign substances, including drugs, from the surface of the eye.[3, 4] Due to these physiological and anatomical barriers, only a very small fraction of the drug, usually 1-5% or even less of the instilled dose, is effectively absorbed.[5] Moreover, the anatomy, physiology and barrier function of the cornea compromise the rapid absorption of drugs. Frequent instillations of eye drops are necessary to maintain a therapeutic drug level in the tear film or at the site of action. But the frequent use of highly concentrated solutions may induce toxic side effects and cellular damage at the ocular surface. To enhance the amount of active substance reaching the target tissue or exerting a local effect in the cul-de-sac, the residence time of the drug in the tear film should be lengthened to improve patient compliance.

Glaucoma affects about 60.5 million people, leaving 8.2 million with bilateral blindness [6, 9]. It is in fact the second largest cause of blindness in the world after cataract.[7] Glaucoma leads to elevated intraocular pressure (IOP) in the eye and degeneration of the axons of retinal ganglion cells (RGCs), which leads to loss of vision and potentially to blindness if not treated. Glaucoma is commonly managed by delivering medications that can mitigate the ocular hypertension, that is, sustained elevation of IOP above 21 mmHg in human.[8,9] Timolol maleate is widely used as a topically applied β-adrenergic blocking agent in ophthalmology to lower the intraocular pressure of glaucoma patients. Due to its systemic absorption timolol may produce systemic cardiac, respiratory, and central nervous system side effects especially in patients with predisposing factors.[10] These side effects have been partly attributed to the poor bioavailability of topically applied ophthalmic drugs and hence it is important to minimize the systemic

concentration of timolol maleate with suitable ophthalmic dosage form design. From the point of view of patient acceptability, a liquid dosage form that can sustain drug release and remain in contact with the cornea of the eye for extended periods of time is ideal. If the precorneal residence time of a drug could be ameliorated from 5 min to one or two hours, then improved local bioavailability, reduced dose concentrations, less total drug, improved patient acceptability and reduced dosing frequency may be achieved. To furnish these properties, delivery systems based on the concept of viscous eye drops can be developed. [10]

Because of the ability of bioadhesive polymers to enhance the viscosity of the ophthalmic vehicles, they are able to reduce the drainage rate of the drugs and subsequently to increase their therapeutic efficacy. Most bioadhesives studied for drug delivery adhere to epithelial tissue and possibly to the mucosal surface of these tissues. One currently accepted mechanism to explain the attachment of some polymers to mucin is a physical entanglement of the polymer chains with mucin when the polymer undergoes swelling in water. [11] In this study, the ocular residence time of Timolol Maleate has been improved by using Hydroxy propyl methyl cellulose (HPMC) K4M and CARBOPOL (CP) 940 as the bioadhesive polymer.HPMC have film-forming properties and specifically, is able to interact with the tear film, increasing its stability.[11]

Viscous eye drops provides the better housing to the delivery system to circumvent the protective barriers like drainage, lacrimation and diversion of exogenous chemicals into the systemic circulation by the conjunctiva. It increases the ocular bioavailability of drug by increasing corneal contact time through effective coating or adherence to corneal surface so that the released drug effectively reaches the anterior chamber. To provide comfort and compliance to the patient and yet improve the therapeutic performance of the drug over conventional systems.[12]

In light of above all facts, main objective of present investigation was to prepare viscous eye drop of TM. Moreover, drug-excipient compatibility study was conducted using differential scanning calorimeter (DSC) and Fourier Transform Infrared Spectroscopy (FTIR). Viscosity of formulation was enhanced by addition of HPMC K4M and CARBOPOL 940. The formulations were characterized to various parameters such as pH, mucoadhasion, viscosity, osmolarity, and in-vitro drug release study. Pharmacodynamic study was also conducted on rabbits and product was compared to marketed formulation.

2. MATERIALS AND METHODS

2.1. Materials

Timolol Maleate was gifted by Marck Bioscience Ltd, Kheda. Hydroxy propyl methyl cellulose (HPMC K4M), Mucin and Poly vinyl pyrrolidone (PVP K30) was procured from Himedia Laboratories Pvt. Ltd. Carbopol 940 was received from S.D. Fine chemicals, Mumbai. Benzalkonium chloride was obtained from Sigma-Aldrich Co. Sodium chloride (NaCl)**,** Sodium Hydroxide (NaOH)**,** Di-sodium hydrogen phosphate (Na_2HPO_4), Potassium Di-hydrogen phosphate (KH_2PO_4), Sodium bicarbonate ($NaHCO_3$), Calcium chloride ($CaCl_2 . 2H_2O$), Sodium Di-hydrogen Phosphate (NaH_2PO_4) and Potassium Chloride (KCl) were gifted by Marck Bioscience Ltd, Kheda. Deionized double-distilled water is used throughout the study.

2.2. Drug polymer compatibility studies

2.2.1 Differential Scanning Calorimetric (DSC)[14]

Differential scanning calorimeter (DSC) was performed using Perkin Elmer instruments, (Perkin Elmer DSC-7, Norway, USA.) to study the thermal behavior of TM and polymers HPMC K4M, CP 940 and mixture of drug and polymers.

2.2.2 Fourier Transform Infrared Spectroscopy (FTIR)[15]

The Infra-Red spectra of Timolol Maleate, HPMC K4M, Carbopol 940 and physical mixtures were conducted using Fourier Transform Infrared Spectrophotometer (Perkin Elmer – spectrum Bx, USA). The procedure consisted of dispersing a sample (drug alone, polymers alone i.e. HPMC K4M, CP 940 and mixture of drug and polymers) in KBr to prepare 10% of mixture and ground generally in mortar-pestle with KBr before being compressed into pellets. This pellet was placed in light path and spectrum was recorded at a resolution of 2 cm^{-1} over a frequency range of 4000 to 400 cm^{-1}. The background spectrum of KBr was used as blank for determination.

2.3. Preparation of Timolol Maleate Viscous Eye Drops

HPMC K4M and Carbopol 940 were dispersed in 50 % of hot water for injection (WFI) as per table 1. The dispersion was vigorously stirred at 800 rpm until dissolved completely. Timolol (0.5%w/v drug) was dissolved in swollen polymeric solution with constant stirring on magnetic stirrer. NaCl was added to adjust osmolarity in range of 290 to 350 mOsm/l. The pH of the dispersion was then adjusted to 7.4 with 10 M NaOH. The amount of benzylkonium chloride and disodium EDTA were added in 0.01 % and 0.1 % respectively. Finally water was added up to 100 ml. (Table 1.) The dispersion was heated in an autoclave in sealed containers to 121^0 C for 20 min. The dispersion was allowed to cool to room temperature (22–23 ^{0}C). The criterion for selection of optimum formula was primarily based on viscosity (25-100 cp), mucoadhesion index

(1000-4877 cp), and cumulative percentage drug release (> 80%).

Table 1. Formulation compositions for viscous eye drops

Ingredients	X1	X2	X3	X4	X5	X6	X7	X8	X9
TM	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68
HPMC K4M	0.5	1	1.5	-	-	-	0.25	0.5	0.75
CP 940	-	-	-	0.2	0.3	0.4	0.225	0.15	0.075
NaOH	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
WFI	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

2.4. Characterization of the Prepared Timolol Maleate Viscous Eye Drops

2.4.1 Clarity[16]

Clarity test was done by visual inspection of each container under a good light, baffled against reflection into the eyes and viewed against a black and white background, with the contents set in motion with a swirling action.

2.4.2 pH[11]

pH of prepared viscous eye drops and marketed formulation was measured with pH meter.

2.4.3 Viscosity[18]

Brookfield Viscometer (LVDV- II + pro, Germany) was used to measure viscosity of prepared viscous eye drops. The thixotropic behaviour was studied from the graphs of Viscosity vs. Shear rate. The viscosity of marketed formulation was also measured for comparison.

2.4.4 Osmolarity

The Osmolarity of sterilized viscous eye drops was determined using freezing point osmometer. 20µl sample was placed in osmometer at 18 ºC for 90 sec test time to measure the Osmolarity.

2.4.5Mucoadhesion Strength [19]

Mucin Dispersion (MUC) (16% w/v) was prepared by dispersing required amount of mucin powder into stimulated tear fluid and kept on magnetic stirrer at 600 rpm for 24 h. for complete hydration. To study the mucoadhesive interaction, 50 ml of polymer dispersion and 50 ml of MUC were mixed. An interaction between polymer and mucin should be seen as a synergistic effect in the rheological properties, which means that the rheological response of the Polymer/MUC mixture should be larger than the sum of the rheological responses of the single components polymer and mucin. Therefore, it is essential to rheologicallycharacterize the single components as well as the polymer/MUC mixture. The graphs of Viscosity vs. Shear rate were plotted for comparison.

2.4.6 Sterility Testing[18]

The sterility testing of the viscous eye drops was performed for the aerobic & anaerobic bacteria by using soya bean casein digest media and alternative thioglycolate media respectively. Incubation was carried out (37 ºC for bacteria and 25 ºC for fungi) and at the interval of every 7 day for 28 days; these media were checked for growth of microorganism by visual turbidity.

2.4.7 In Vitro Drug Release (Diffusion Study)[4, 18]

The Franz diffusion cell was used for measuring *in vitro* drug release of the viscous eye drops. A cellulose acetate membrane (Dialysis membrane with 25 mm diameter) was adapted to the terminal portion of the cylindrical donor compartment. 2.5 ml viscous eye drops containing drug, sufficient for establishing sink conditions for the assay was placed into the donor compartment. The receptor compartment contained 20 ml of Phosphate buffer solution (pH 7.4) maintained at 37 °C ± 0.5 ºC under mild agitation using a magnetic stirrer. One ml of sample was withdrawn at predetermine time interval of every h. for 12 h. and immediately restored with the same volume of fresh phosphate buffer (pH 7.4). This aliquot was filtered and diluted with phosphate buffer (pH 7.4). The amount of drug released was analyzed in Shimadzu U.V. Spectrophotometer at 294 nm. The drug release profile of all batches was fitted into a zero-order, first order, Higuchi, Hixon-Crowell cube root law and Korsmeyer-Peppas to access the kinetic modelling of drug release.

2.5 *In Vivo* studies

The optimized formula was evaluated for *in vivo* performance in animal model (New Zealand Male Albino Rabbits). The preclinical experimental protocol (Protocol no. 1107) was approved by college ethics committee (Ethical committee registration number is 277/CPCSEA). Eighteen rabbits (New Zealand Male Albino Rabbits) were used for this study. They were housed and maintained in the animal house at room temperature (27 ºC) during the period of the study. They were fed with standard diet and water.

2.5.1 Anti-glaucoma activity by measurement of intra ocular pressure (IOP)[18]

Twelve animals were randomly divided into groups containing 4 animals in each group. Group I having glaucoma only, Group II and III for disease plus treatment with optimized formula of viscous eye drops and marketed respectively. Glaucoma disease was induced by Bonomi *et al* method. Twelve rabbits were treated with subconjunctival injections of 0.25 ml Betnesol injection (Betamethasone sodium 4 mg/ml) every week for four weeks in left eye (fig. 1). Right eye was kept as control and treated with 0.25 ml normal

saline solution. Local anaesthetic propracaine eye drops were used prior to subconjunctival injection. The activity was confirmed by noticing a bulge formation at the site of injection. [15] The right eye of each rabbit was kept as control for glaucoma and the left eye was treated for glaucoma using optimized eye drops and marketed formulation for group II and group III respectively. The IOP readings were measured using Schiotz Tonometer (fig. 2) at every 30min, 1st, 2nd, 3rd, 8th, 12th and 24th after drug administration. All IOP measurement data was analysed with two-way ANOVA. A P value of 0.05 or less was considered significant.

Table 2.Groups and Treatments of Animals for Anti glaucoma activity

Group	Treatment
Group I	Glaucoma only
Group II	Disease plus drug administer via optimized viscous eye drops. (Test)
Group III	Disease plus Marketed formulation (Standard)

FIGURES

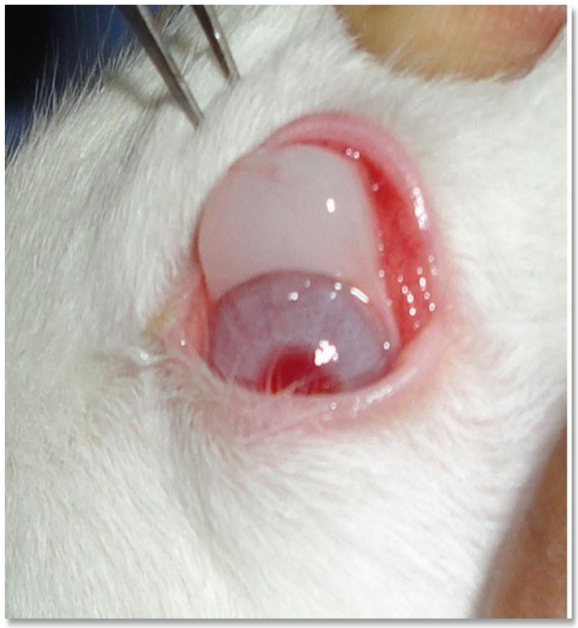

Figure 1 Bulge formations at the site of action

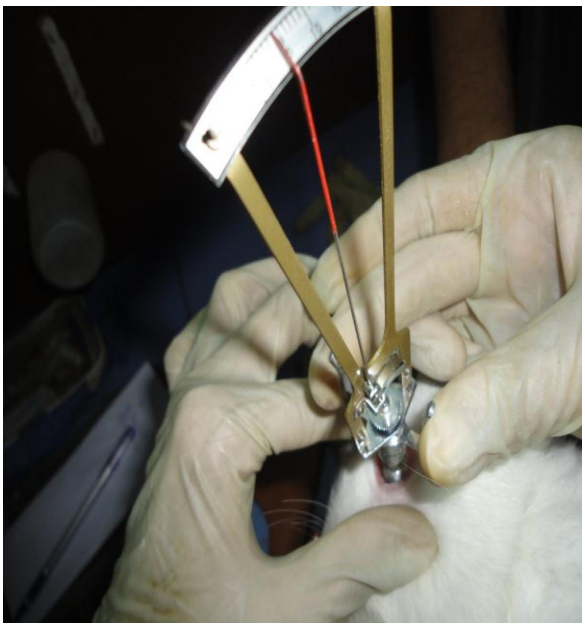

Figure: 2. Measurement of IOP in rabbit eye using Tonometer

2.5.2 Ocular Irritation study [2]

Six animals were divided into 2 groups having three animals in each for ocular irritation study. Group I with control and Group II was administer drug via viscous eye drops. Ocular irritation study was performed according to Draize technique on six male albino rabbits. The optimized formula was instilled daily for a period of 21 days and the rabbits were observed for redness, swelling, watering of the eye.

2.6 Stability

A short term stability study of optimized formula was done at specified temperature and humidity (40±1 ^{0}C and 75 % RH) according to ICH guidelines. The chemical stability of the formulation was assessed by the estimation of the percentage drug content and physical stability was evaluated by change in pH, viscosity and appearance.

3. RESULTS AND DISCUSSION

3.1. Drug - Polymer Compatibility Studies

3.1.1. DSC

The Differential Scanning Calorimetric (DSC) curve of Timolol maleate [Fig. 3 (a)] displays a single sharp endothermic peak at 204.86 ^{o}C corresponding to its melting point. [20] DSC spectra of HPMC K4M and CP 940 are shown in figure 3 (b) and (c). The DSC peak of physical mixture 1 of TM and HPMC K4M [Fig. 3 (d)] and that of physical mixture 2 of TM and Carbopol 940 [Fig. 3 (e)] shows similar endothermic peak to pure component but less intense. This may be due to smaller concentration of TM selected did not interfere with TM nor they make any shift in melting point. In Physical mixture 2 due to overlapping peaks of TM and CP 940 peak broadening is observed in the range of 200 ^{o}C to 210 ^{o}C. A similar endothermic peak of physical mixtures and pure components confirms no significant interaction between drug and polymers.

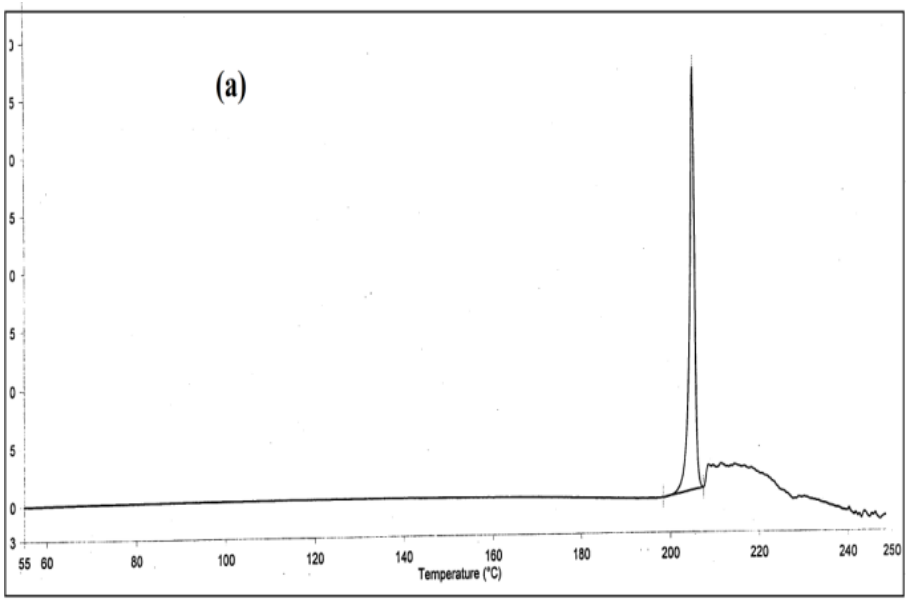

Figure: 3 (a) DSC thermogram of Timolol Maleate

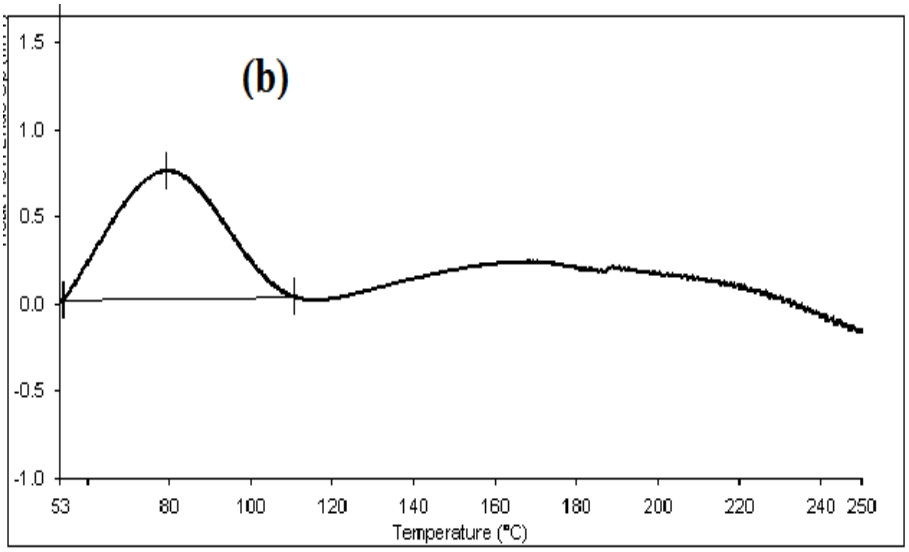

Figure: 3 (b) DSC thermogram of HPMC K4M

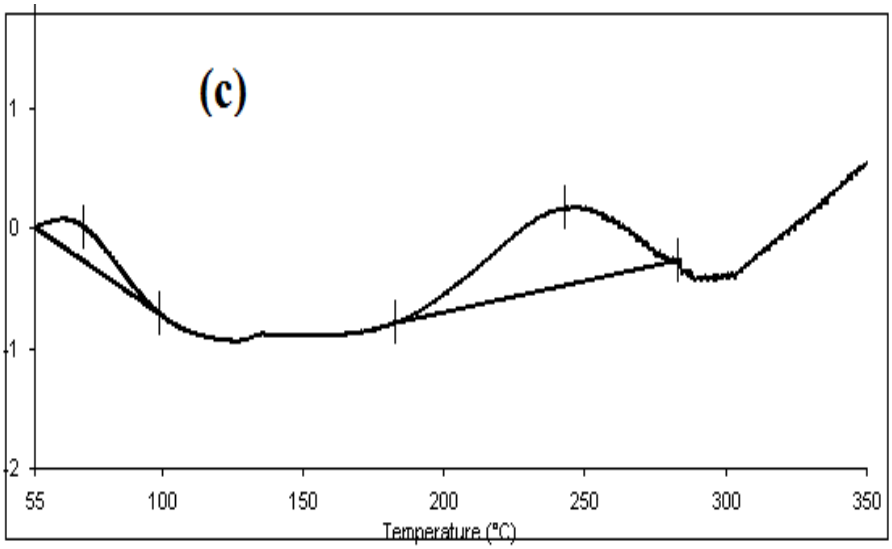

Figure 3 (c) DSC thermogram of CP 940

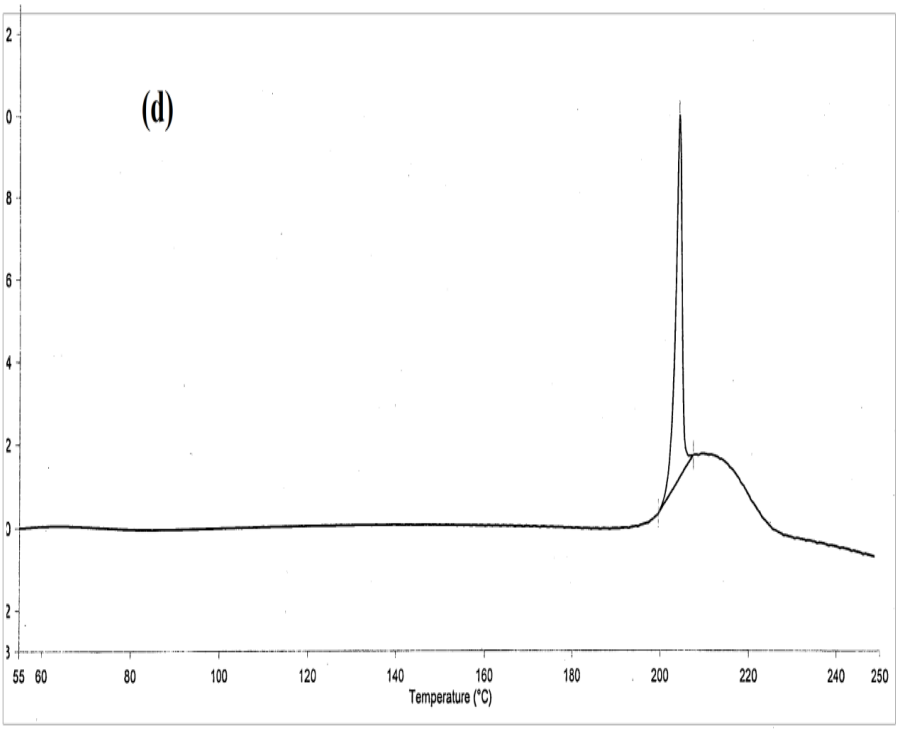

Figure 3 (d) DSC thermogram of Physical mixture 1: Timolol Maleate and HPMC K4M

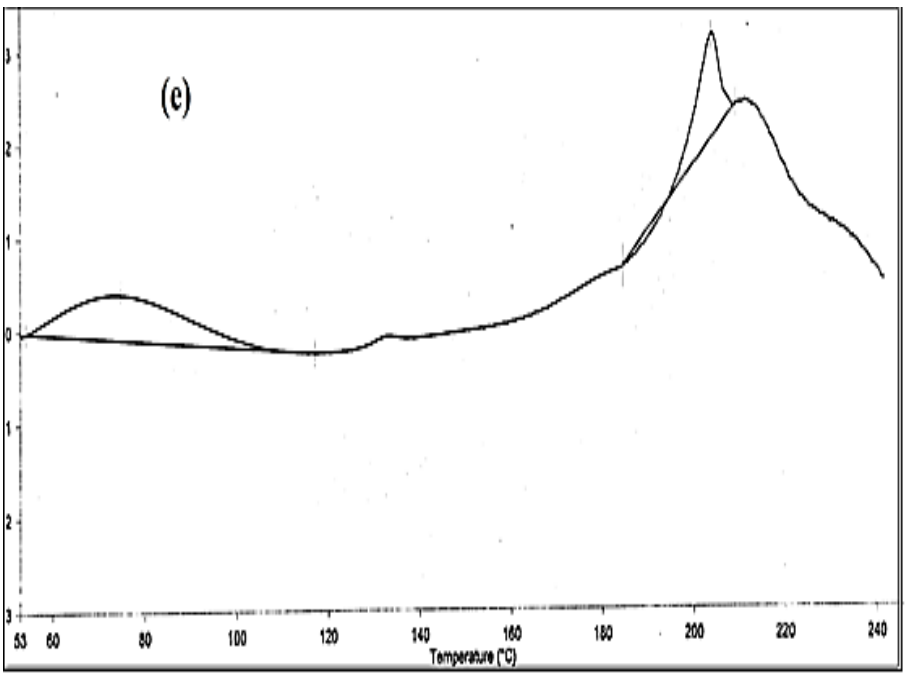

Figure 3 (e) DSC thermogram of Physical mixture 2: Timolol Maleate and Carbopol 940.

3.1.2 FTIR

Figure 4 (a) depicts FTIR spectra of pure Timolol maleate with principal peaks at 2966 and 2855cm^{-1} indicating the presence of –OH group. The absorption due to the –NH group present molecule is supported by exhibition of a shoulder to the main peak around 1711 and 1494 cm^{-1}. The drug contain more than one C=N absorption present in the thiadizole moiety of the heterocyclic ring system.[20] Figure 4 (b) and 4 (c) shows FTIR spectra of HPMC and CP 940 respectively. Infrared Spectra of Timolol Maleate, Physical mixture 1 and Physical mixture 2 [Fig 4 (d) and 4 (e)] revealed no considerable changes in the FTIR peaks of Timolol Maleate in the physical mixtures in both cases when compared to pure drug thereby indicating the absence of any interaction at physical mixture level.

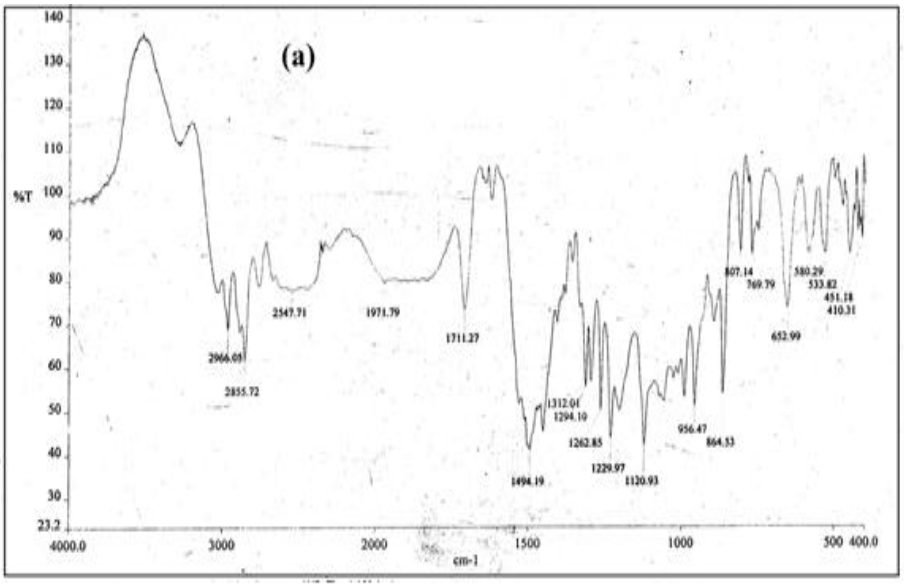

Figure 4 (a) FTIR spectra of pure Timolol Maleate

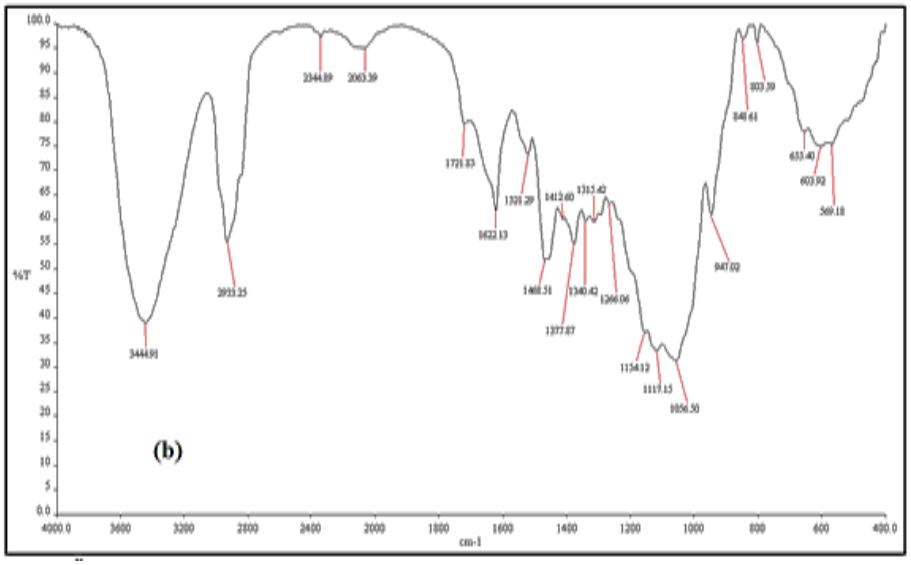

Figure 4 (b) FTIR spectra of pure HPMC K4M

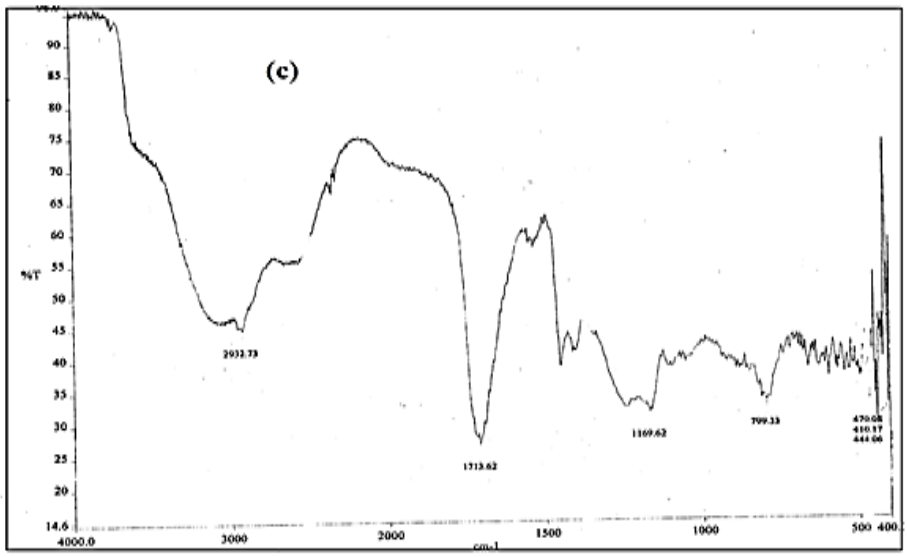

Figure 4 (c) FTIR spectra of pure CP 940

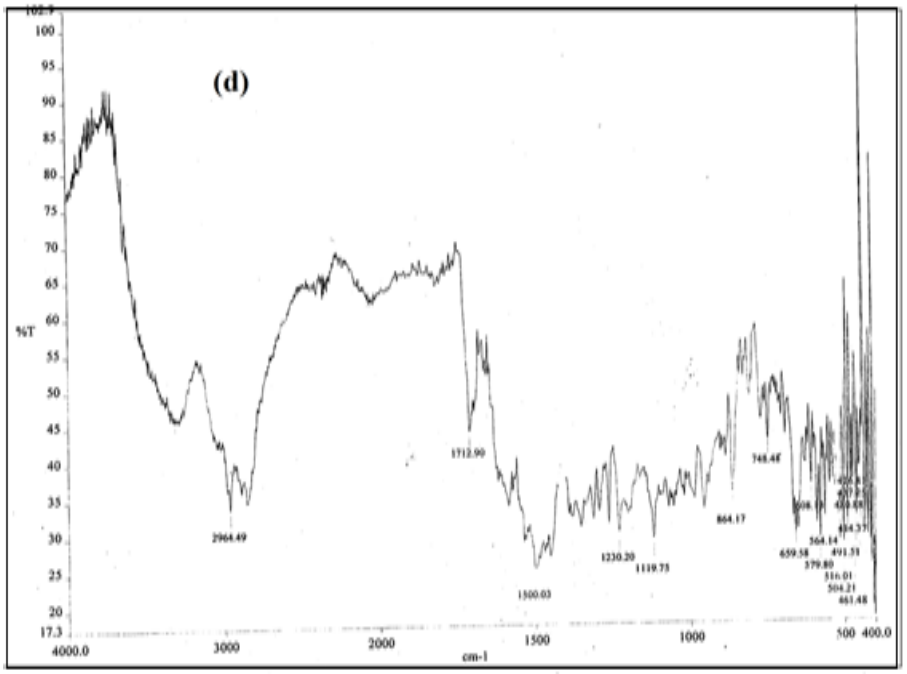

Figure 4 (d) FTIR spectra of Physical mixture - 1 With Timolol Maleate and HPMC K4M

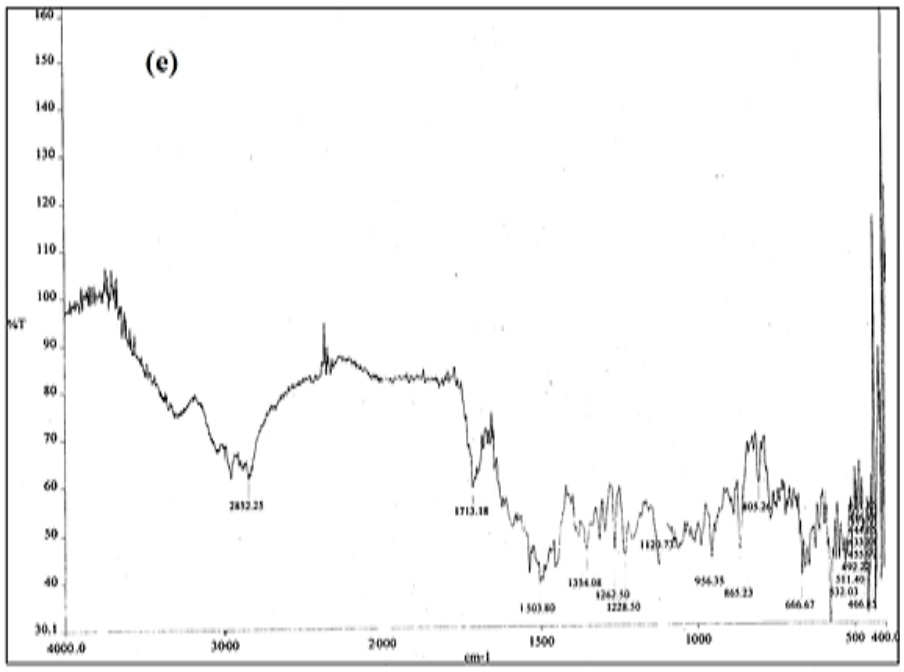

Figure 4 (e) FTIR spectra of Physical mixture - 2 With Timolol Maleate, HPMC K4M and CP 940.

3.2. Physical Characterization, pH, Osmolarity and Assays of Timolol Maleate Viscous Eye Drops

3.2.1 Clarity

Clarity of the batches X1 to X3 having HPMC K4M was found to be clear. As the concentration of CP 940 increase, the opacity was increase and it became translucent. Clarity of solutions depends upon the concentration of polymer. As the concentration of CP 940 increased, solutions became translucent.

3.2.2 pH

pH of all the batches were found nearer to 7.4 in all triplicate observations and they complies with the acceptable range of ophthalmic preparation.

3.2.3 Viscosity

From the rheograms shown in figure (5), batches X1 and X4 shows very low viscosity due to lowest concentrations of 0.5 % HPMC and 0.2 % CP 940 respectively; was not selected as the aim of this study was to increase the viscosity of the formulations

developed for residence time in ocular globe.[24] Batch X3 was too viscous to instill properly into the eye. The entire batches showed non-newtonian (dilatant) flow behaviour without any hysteresis. Non-newtonian solutions offer less resistance to movement of the eyelids over the globe and, therefore, are expected to be more comfortable in the eye than newtonian solutions.[11] By using the viscolysers like HPMC and CP 940, viscosity of the prepared eye drops was increased which gives longer contact time to the ocular globe. Therefore frequency of the instillation is decreased, which improves patient compliance.[23] When viscosity results of the pre-formulation study of 0.5%, 1% and 1.5% w/v HPMC solutions were compared to viscous eye drops formulations, it could be deduced that the addition of drug plus excipients to the solutions caused an increase in viscosity because the HPMC solutions caused an increase in viscosity of the solutions due to the presence of hydrogen-bonding of amino groups of the TM to HPMC molecules.[24]

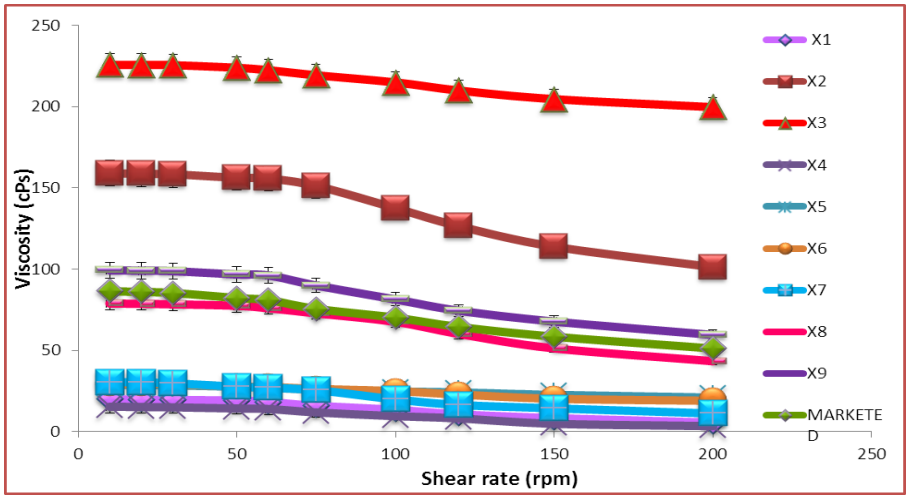

Figure: 5 Rheograms of Viscosity vs. Shear rate of all batches compared with Marketed

3.2.4Mucoadhesion Test

Mucoadhesion test of the batches X1 to X3 containing only HPMC in different concentrations was exhibited very poor mucoadhesion strength due to non-ionic nature and exhibited poor bond formation with mucin. As CP 940 is anionic in nature, it form bond with mucin and gives good mucoadhesion strength.[1] In a neutral medium, the mucin molecule is negatively charged (pKa-2.6) and behaves as an anionic polyelectrolyte, forming a weak viscoelastic gel, which consists of a network of linear, flexible and random coil molecules. Polymer–mucin interactions include chain interlocking, conformational changes and non-covalent bond formation. Polymers should, therefore, have functional groups that are able to form hydrogen bonds and the polymer chain should be flexible enough to form as many bonds as possible.[25] In combination of HPMC and CP 940, batches X7 to X9 showed excellent mucoadhesion strength and higher viscosity of solutions due to HPMC was contributed for viscosity while CP 940 was responsible for mucoadhesion strength. As shown in figure (6), batches X7 to X9 complied with the results of Marketed formulation and showed excellent mucoadhesion strength.

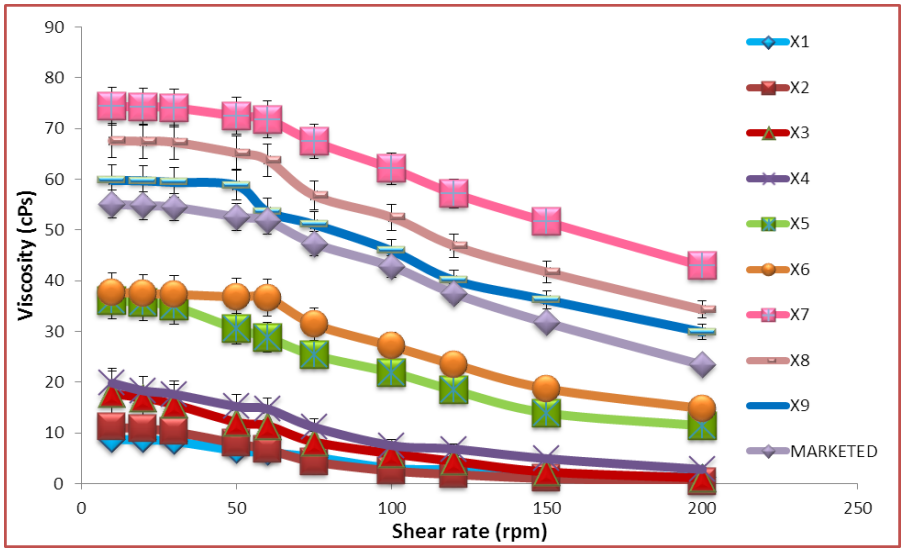

Figure 6. Rheograms of Viscosity vs. Shear rate for Mucoadhesion Test

3.2.3 Osmolarity

The Osmolarity of the ophthalmic preparation should be in the range of 310-350 mOsmol/kg to avoid irritation. [11] Osmolarity of all batches was found to be in the range which was within the acceptable tonicity range of the eye to avoid ocular irritation.

3.2.4 Assay of Timolol Maleate and Benzalkonium Chloride

All the prepared formulations complied with the pharmacopoeial limits for content assay of TM and BKC.

3.3. *In Vitro* Drug Release and Kinetics of Timolol Maleate from the Prepared Viscous Eye Drops

In the development of viscous eye drops, a drug release testing is very important to assure batch-to-batch uniformity of each drug delivery system and to evaluate the release rate of the drug from the prepared formulae. The drug release data from viscous eye drops containing HPMC K4M alone clearly indicated that the release of the drug was influenced by the concentration of the HPMC K4M. The viscosity of HPMC K4M was an important factor and affects the release behavior of the drug from viscous eye drops. The dissolution results are shown in Fig. (7). It indicated that optimized formulations gave sustained release effects as compare to marketed formulation. Drug release up to 40% was occurred in first two hours. The rate and extent of drug release was decreased with increasing the amount of HPMC K4M. Percentage cumulative drug release (% CDR) of all batches was calculated for the diffusion co-efficient showed the penetration of drugs through the intact cornea is not a matter of simple diffusion but is best represented by the concept of differential solubility. In present study, the diffusivity was not affected by polymers. From these result, it is clear that this improvement is due to increased partitioning of TM to the corneal epithelium.

Linear regression analysis for the release data was done to determine the proper order of release. Zero-order, first-order, Higuchi and Korsmeyer Peppas model equations were applied to all in vitro release results. From the results shown in Table 3, it could be concluded that the drug is released by a non-fickian diffusion mechanism from all the viscous eye drops.

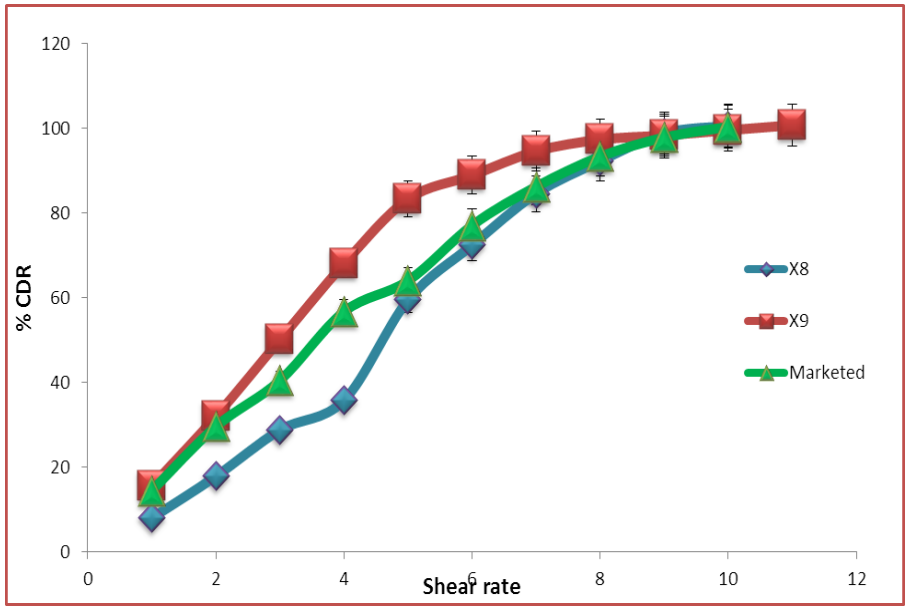

Figure: 7 Comparison of release behavior of optimized batch with Marketed

Table: 3 Fitting of drug release kinetics models for all batches and Marketed formulation

BATCH	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	K	R^2	K	R^2	K	R^2	N	R^2
X1	4.74	0.632	0.036	0.524	0.286	0.817	0.937	0.999
X2	4.25	0.565	0.354	0.457	0.279	0.769	0.831	0.996
X3	4.65	0.693	0.057	0.579	0.333	0.834	0.934	0.935
X4	4.01	0.636	0.029	0.527	0.256	0.83	0.759	0.998
X5	3.80	0.565	0.031	0.456	0.267	0.773	0.999	0.999
X6	4.49	0.694	0.049	0.554	0.315	0.852	0.695	0.976
X7	4.89	0.646	0.043	0.501	0.300	0.83	0.823	0.995
X8	4.71	0.605	0.041	0.485	0.301	0.798	0.881	0.987
X9	3.46	0.496	0.026	0.392	0.237	0.717	0.654	0.999
Marketed	3.21	0.501	0.034	0.421	0.254	0.811	0.783	0.985

3.4 *In Vivo* Measurement of Intra Ocular Pressure (IOP) to Check Anti-Glaucoma Activity

The optimized batch X9 was analyzed for in vivo studies using rabbits. Results concerning IOP measurements before and after treatment are indicated in table 4 and 5 respectively. The maximal decrease in IOP was obtained 1 h. after instillation for both the treatments (marketed and batch X9). The inhibition of hypertension for test was 23.4%, corresponding to a 2.4 mmHg decrease, statistically comparable with the inhibition obtained marketed, which was 24.1%, corresponding to a 1.6 mmHg decrease. This result is classically described for β-blocker activity in the rabbits. However, the optimized batch X9 significantly extended the duration of the pressure-reducing effect of TM for up to 8 h. after instillation (fig. 8). The optimized batch X9 presented a hypotensive activity of 15.1% inhibition corresponding to a 10.6 mmHg decrease, significantly higher than that of marketed formulation. The in vivo experiments demonstrated a modification of the TM activity in the presence of HPMC and CP 940. The IOP reduction studies demonstrated a significant enhancement of the duration of action of the TM viscous eye drops.

Table: 4. IOP before treatment

Group	R*	L*
Group I	16.9±0.22	25.4±0.07
Group II	17.6±0.14	25.8±0.07
Group III	18.2±0.07	25.7±0.07

* Value Expressed as Mean ± SD (n=4)
R - Right Eye
L - Left Eye

Table: 5 IOP after treatment

Time (hrs.)	Group II (Test)		Group III (Std.)	
	R*	L*	R*	L*
0.5	19.1±0.17	23.4±0.11	18.5±0.03	24.1±0.11
1	19.1±0.17	22.5±0.07	18.5±0.03	23.8±0.09
2	19.1±0.17	21.2±0.07	18.5±0.03	22.5±0.07
3	19.1±0.17	18.6±0.04	18.5±0.03	19.7±0.06
4	19.1±0.17	17.3±0.07	18.5±0.03	18±0.11
6	19.1±0.17	16.2±0.03	18.5±0.03	17.4±0.03
8	19.1±0.17	15.1±0.12	18.5±0.03	16.3±0.09
10	19.1±0.17	15.3±0.03	18.5±0.03	16.6±0.03
12	19.1±0.17	16.2±0.13	18.5±0.03	18.1±0.13
24	19.1±0.17	19.6±0.07	18.5±0.03	20.2±0.14

*Value Expressed as Mean ± SD (n=3)

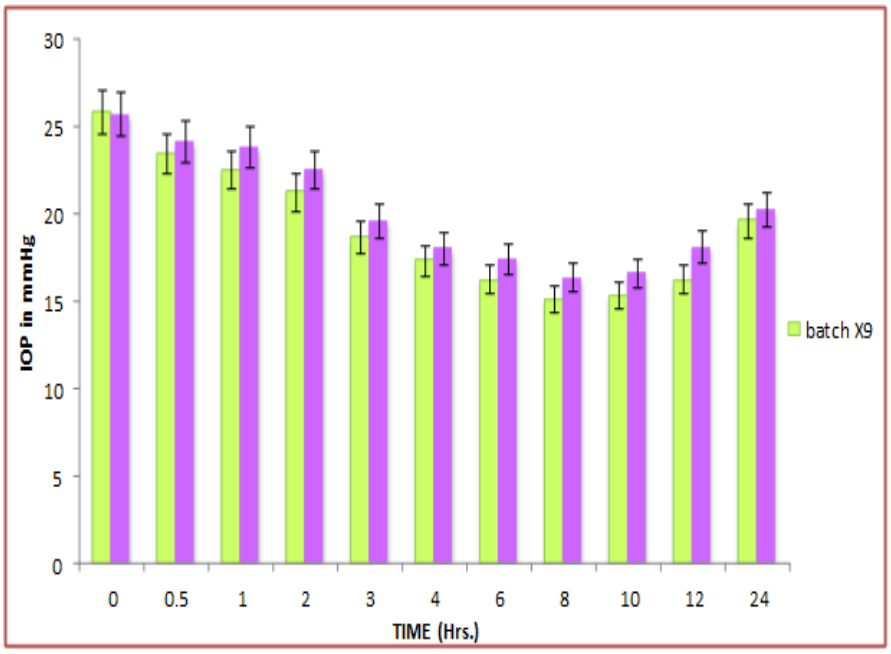

Figure: 8 Comparison studies of viscous eye drops with Marketed for IOP measurement

3.4.1 Statistical Analysis

All IOP measurement data was analysed with ANOVA: Two-Factor without Replication. A P value was found to be 0.00048 that was considered significant as less than 0.05.

3.5 Ocular Irritation Study

The optimized Batch X9 was found to be non-irritating with no ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae, redness and swelling observed in all four rabbits. Thus Batch X9 is safe, provides therapeutically efficacious and suitable for the eye instillation.

3.6 Stability Study

A stability study data indicates absence of any degradation of the formulation over six months. Also from t-Test: Two-Sample Assuming Equal Variances, it was conformed that there absence of significant difference in initial reading and after 6th month stability data.

Table: 6. Change in pH, Assay of Timolol Maleate and Assay of BKC data over time (Stability Study)

Month	pH*	Assay of Timolol Maleate (% w/v)*	Assay of BKC (% w/v)*
1	7.4 ± 0.01	102.69±0.10	100.12±0.14
2	7.41 ± 0.02	101.47±0.11	100.01±0.13
3	7.4 ± 0.01	100.97±021	99.97±0.2

*Value Expressed as Mean ± SD (n=3)

CONCLUSION

The results of this study reveal that the higher concentration of CP 940 was responsible for translucent appearance of solutions. Each batch showed non-newtonian (dilatant) flow behavior without any hysteresis. In combination of HPMC K4M and CP 940, batches X7 to X9 shows excellent mucoadhesion strength because of higher viscosity of solutions due to HPMC K4M as well as Carbopol 940 which is responsible for mucoadhesion strength. Osmolarity, pH and assay of TM and BKC values was in the pharmacopoeial acceptable range. The present investigation suggests that the drug release from all formulations follows Korsmeyer-Peppas kinetics and the higher rates were achieved from batch X9 within 11 hours as compared to the other patches. The results of the animal experiments clearly demonstrated that the optimized batch X9 showed no irritation and provided a better sustained release of the drug in comparison to the marketed dosage form lasted for 12 h. Stability studies revealed that there was no decomposition of any formulation over six months. Thus the formulation may be considered as a promising delivery for ophthalmic use of timolol maleate.

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