ISSN (Online) 2249-6084 (Print) 2250-1029

International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)
[Impact Factor – 0.7826]

Journal Homepage: www.eijppr.com

Research Article

Design and Evaluation of *In Situ* Gelling Systems of Moxifloxacin Hydrochloride in the Treatment of Bacterial Eye Infections

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Article info

Article History:
Received 16 September 2013
Accepted 30 December 2013

Keywords:
Moxifloxacin, Bacterial eye infections, *In situ* gel, Carbopol 980

Abstract

Eye is a very sensitive organ and cannot withstand high local concentrations of drugs or vehicles without irritation, and as such designing formulations and delivery systems for topically applied ophthalmic drugs is challenging. The short precorneal residence time results in low bioavailability and frequent dosing is usually needed to compensate for the loss of drug. For patient acceptability, a liquid dosage form that can sustain drug release and remain in contact with the cornea of the eye for extended period of time is ideal. The present study is aimed at formulating a novel in situ gelling system of Moxifloxacin hydrochloride, based on the polymer – Carbopol 980 with added viscolisers to overcome the drawbacks of conventional eye drops and provide novel assets like, reduction in the frequency of administration, prolonged delivery of drug, high drug bioavailability and ease of administration. An experimental design of four formulations was set up. The developed formulations were found to be stable, non-irritant, and efficacious. In-vitro drug release profile indicated sustained release of drug over a period of 12 hours in simulated tear fluid. The developed formulations were found to be viable alternatives to conventional eye drops fulfilling the requirements of ideal control release ocular delivery system, for twice a day therapy, by virtue of their ability to sustain drug release over a 12 hour period.

1. INTRODUCTION

The poor accessibility of a number of ocular regions to systemic circulation makes local delivery via topical administration the preferred route for the treatment of ocular diseases. Typical conditions that require ocular administration include eye infections (i.e., conjunctivitis) and corneal disorders (i.e., glaucoma). The biological barriers involved for ocular delivery are the permeability barriers posed by cornea and other regions, as well as the tear washout and blinking reflexes designed to remove foreign substances from the eye.

A thorough understanding of physiological basis of the protective mechanism designed by the eye which allows only 1-10% of topically applied dose to be absorbed locally.

Attempts to improve ocular bioavailability have been focused for overcoming precorneal solution drainage. The ocular drug delivery systems, while limited in providing ideal bioavailability profiles, do provide opportunities for improvement[1].

Thus, eye presents a challenge in development of sustained or controlled release systems which could be used to prolong corneal contact time, thus increase the bioavailability and at the same time preserve visual acuity[2]. Such systems should be more hydrophobic, minimize interference with blinking and exhibit pseudo plastic behavior. A better approach of ocular product behavior coupled with formulation optimization can lead the way to development of newer ocular drug delivery systems[1].

2. MATERIALS AND METHOD

The materials used include Moxifloxacin Hydrochloride (gift sample from Cipla Ltd Verna – Goa), Carbopol980 (gift sample from Noveon Carbopols Mumbai), Hydroxy Propyl MethylCellulose (gift sample from Colorcon Asia Verna - Goa), Hydroxy Ethyl Cellulose(gift sample from Aventis Pharma Verna - Goa) , Polyvinyl Alcohol(procured from B.D.H. Chemicals), Benzalkonium Chloride(gift sample from Centaur Pharmaceuticals Karaswada - Mapusa), Sodium Chloride(procured from Hi Media Chemicals), Potassium dihydrogen ortho phosphate, Disodium hydrogen Phosphate, Sodium Hydroxide of Analytical grade were procured from Loba Chemie pvt Ltd Mumbai.

2.1 Preformulation Study

2.1.1 Selection of Vehicle

Solubility of Moxifloxacin was tested in distilled water, citrophosphate buffer and phosphate buffer. Moxifloxacin Hydrochloride was found to be soluble in distilled water and freely soluble in phosphate buffer (pH 6, 6.8, 7.4) and formed clear solutions, while was soluble in citrophosphate buffer also, but resulted in translucent solutions. Further aqueous solution of Carbopol 980 in distilled water and phosphate buffer formed transparent sols while that of citrophosphate buffer formed cloudy sols.

Aqueous solutions of carbopol 980 are low viscosity acidic solutions that transform into gels with an increase in pH and hence are advocated as in situ gelling ophthalmic systems. Phosphate buffer of pH 6.8 was selected as vehicle as it formed sols having pH value close to pH 6 as using distilled water resulted acidic solutions having pH value less than pH 5, and citrophosphate buffer lead to cloudy sols .

2.1.2 In situ gelling Concentration

Aqueous solutions of carbopol in varying concentration ranging from 0.1-1% were prepared and tested for gelling capacity. Gelling capacity was determined by placing a drop of the system in a vial containing 2ml of artificial tear fluid (phosphate buffer pH 7.4) freshly prepared and equilibrated to 37 º C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve.

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The study was carried out using combination of gelling agent and viscolisers in various combinations where in a combination of 0.4% of carbopol 980 with 0.5% viscoliser yielded the best result i.e. low viscosity solutions with immediate gelling and for an extended period of time.
When used alone the optimum concentration of Carbopol 980 for forming in situ gels was found to be 0.7% which formed low viscosity acidic solution. When used in combination 0.4% of carbopol 980 with 0.5% individual viscoliser (HPMC K15, HEC, PVA) yielded the best result i.e. low viscosity solutions with immediate gelling and for an extended period of time. The results for which are stated in table-1.

2.2 Formulation Development
The type and concentration of the three most commonly added viscolisers; HPMC, HEC and PVA is taken up in the study with Moxifloxacin hydrochloride(0.5%w/v) and Carbopol 980 as the main gel forming polymer in the concentration of 0.4%w/v along with 0.5%w/v concentration of the viscolisers respectively. Benzalkonium chloride has been shown to be the most effective and rapidly acting preservative 0.01% benzalkonium chloride was selected as the preservative.
To find out the influence of viscoliser, namely HPMC, HEC, PVA an experimental design of four formulations was set up and is as presented in the table-2.

2.2.1 Preparation of the Formulation
The detailed procedure for preparing the in situ gelling systems of Moxifloxacin hydrochloride is as outlined below:
The buffer salts were dissolved in 75ml of purified water. The viscolisers were added and allowed to hydrate. Carbopol was sprinkled over this solution and allowed to hydrate overnight, followed by stirring with an overhead stirrer. Moxifloxacin hydrochloride was dissolved in sodium hydroxide solution. Benzalkonium chloride (BKC) and sodium chloride were then added and the solution was filtered through 0.22 micron Millipore membrane filter. The drug solution was added to the carbopol – viscoliser solution under constant stirring until a uniform solution was obtained. Phosphate buffer was then added to make up the volume to 100ml.
The developed formulations were filled in 5ml capacity amber glass vials, closed with grey butyl rubber closures and sealed with aluminium caps. The formulations, in their final packs were subjected to terminal sterilization by autoclaving at 121 º C and 15 p.s.i. for 20 minutes[3].

2.3 Evaluation
The devised Sol Gel formulations of Moxifloxacin hydrochloride based on Carbopol were evaluated for clarity and appearance, pH, viscosity and gelling capacity, drug content uniformity, compatibility studies(Solid and Liquid state), in-vitro studies, microbiological evaluation, ocular irritancy study and stability study.
The clarity and appearance of the developed formulations were checked against black and white background using Clarity apparatus-Visual board.
Measurement of pH was carried out for sols and the gels (obtained by neutralizing sols to gels with phosphate buffer pH 7.4) at room temperature with the help of a pH meter.
The two main prerequisites of a gelling system are Viscosity and Gelling capacity (speed and extent of gelation). Measurement of viscosity was done for all treatment combinations of sols and gels (sols were converted into gels by addition of phosphate buffer pH 7.4) using Brookfield viscometer LVT model , fitted with spindle No2 at speed 0.6 rpm at room temperature.
Gelling capacity was determined by placing a drop of the system in a vial containing 2ml of artificial tear fluid (phosphate buffer pH 7.4) freshly prepared and equilibrated to 37 º C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel form to dissolve[3].
To find the drug content uniformity of Moxifloxacin hydrochloride in Sol, spectrophotometric method was followed.1ml of sol was transferred to a 100ml volumetric flask and the volume was made up with phosphate buffer pH 7.2. The absorbance of the resulting solution were determined at λmax 291nm using a suitable reagent blank. The concentration was obtained from the calibration curve. The test was performed in triplicate and the mean standard deviation of variance was calculated The results for pH , gelling capacity ,viscosity , drug content uniformity are tabulated in table-3.
Compatibility studies were conducted in solid state by infra-red spectroscopy and liquid state by high performance thin layer chromatography.

2.3.1 Solid state compatibility study
Infra-red spectrum of all physical mixtures were carried out for the study using FTIR and the spectrum run from 400 to 4000 cm^{-1} to rule out any possible incompatibilities in solid state **.**

2.3.2 Liquid state compatibility study
Solution phase compatibility was established with high performance thin layer chromatography. All the formulations M1 to M4 were chosen for the study keeping in view that this study is decisive factor for the entire project work undertaken. Each formulation was extracted with acetonitrile along with the pure drug solution in acetonitrile, spotted on a silica gel 60GF $_{254}$ plate prewashed with methanol. n-Butanol, Methanol and Ammonia solutions 4:4:2 were used as mobile phase[4]. The distance traveled by solvent front was noted and the spots were visualized in a UV chamber. The distance traveled by solute was also determined. The Rf values of the standard and the sample was computed using the formula given below to establish compatibility.

$$\text{Rf Value} = \frac{\text{Distance traveled by solute from the point of application}}{\text{Distance traveled by solvent front from the point of application}}$$

2.4 *In vitro* Release Study
In-vitro studies were carried out in an in house designed, bi-chambered apparatus using commercial semi-permeable membrane of transparent and regenerated cellulose type Cellophane. Cellophane prior to use, was rinsed well in acetone and soaked in phosphate buffer pH 7.4 overnight and tied to one end of a pyrex cylinder (internal diameter3.61cms), both ends open. 50ml of phosphate buffer pH 7.2 was taken in 100ml recipient compartment and the cylinder was lowered on to the surface of the dissolution medium so as to just touch the surface of the fluid. The dissolution medium was stirred magnetically using magnetic rods, with the help of magnetic stirrers and maintained continuously at 37 ºC. Low speed of 20 rpm was kept constant for all the formulations. 0.7ml of phosphate buffer pH 7.4 was placed in the pyrex cylinder through the open end (which constituted the donor compartment) and 0.5ml of all four formulations were individually placed in donor compartments where they get converted into gels. For the release study, aliquots of 5ml were withdrawn from the release media at periodic intervals and replaced with equal volume of dissolution media[5]. The drug content was analyzed at 291nm against a reference standard using phosphate buffer pH 7.2 as blank on a UV/Visible spectrophotometer.

2.5 Microbiological Study

2.5.1 Sterility Testing
The tests were carried out under conditions designed to avoid accidental contamination of the product during the test. Soyabean Casein Digest Media (SCDM) as fungal media, and Fluid Thioglycollate Broth Media (FTG) as bacterial media, were used in a sterile condition after autoclaving at 121 º C for 20 minutes at 15 p.s.i. For explaining the results of sterility testing, controls were kept. Negative control included sterilized media to check efficiency of sterilization, while positive control included sterilized media inoculated with micro-organisms to tests the sensitivity of the media in supporting microbial growth. The media FTG and SCDM were inoculated with *Staphylococcus Aureus* as the bacterial and *Candida Albicans* as the yeast cultures respectively. The bacteria were incubated at 30 º C to 32 º C for seven days and the yeast at 22 º C to 25 º C for two days. Inactivation of both the drug and preservative was done by dilution after addition of sample to a volume of broth larger than the normal. Thus 1ml of sample was added to 50ml of sterile distilled water and 1ml of the resulting dilutions were added to 10ml of culture media with due care to prevent accidental contamination.
The test containers were incubated at 30 º to 32 º C for bacteria and 22 º to 25 º C for fungi for fourteen days. The containers were inspected after swirling everyday to detect any possible contamination. From the observations, the results were interpreted.

2.5.2 Determination of Minimum inhibitory concentration (MIC)
The tests were carried out by mixing the appropriate volume of the stock solution of Moxifloxacin Hydrochloride with 5ml double strength broth and the volume was made up with sterile distilled water To each of the tubes, 0.1ml of an 18 hour old culture suspension of *Staphylococcus Aureus* was added. The contents of the tube were cyclomixed and incubated at 37 º C for 18 hours. The tubes were examined for the presence or absence of growth (indicated by turbidity of the solution) at the end of 18 hours. The lowest concentration capable of preventing the microbial growth is called the MIC. The concentration of stock solution is equal to 10 µg /ml[6].

2.5.3 In-vitro Effectiveness Studies
In in-vitro effectiveness study was performed by cup plate method using antibiotic assay agar. The plates were prepared by pouring 16ml of sterilized agar on the plate as the base layer. The seed layer was prepared after mixing 18 hour old culture suspension of *Staphylococcus Aureus* and this inoculated media was poured over the surface of the solidified base layer and allowed to settle. Cups were bored into the plates with the help of a sterile cork borer and 0.2ml of formulations were filled in the cups . The plates were incubated at 37 º C for 24 hours and diameter of zones of inhibition was measured. Positive control and Negative controls were also maintained. Positive control included seed agar inoculated with 18 hour old culture suspension of *Staphylococcus Aureus* poured over the base layer .Negative control includes uninoculated seed agar poured over the base layer [6].

2.6 Biological Studies
Biological study was carried out as ocular irritancy study. The objective of this study is to ascertain whether the formulation may cause irritation of the eyes.

2.6.1 Ocular Irritancy Test
It was conducted in the following manner: six healthy male albino rabbits weighing 1-2 kgs were used for the study and given thorough ophthalmologic examination 24hrs before putting them for use, to ensure absence of pre-existing ocular damage. They were kept in separate cages with husk bedding and fed with standard diet and water as much as required. The animals were gently though firmly restrained. The test formulation M2 was placed in the left eye of each animal by carefully pulling the lower eyelid away from the eyeball (conjunctival cul de sac) to form a cup into which 0.1 ml of the formulation was dropped. The eyelids were cautiously held for 1 sec and the animal was released. The right eye was considered as control. The eyes of each rabbit was examined immediately and later 24, 48, 72 hrs after adding of the test formulation[7].
Stability studies were performed at room temperature for three months. Out of the four formulations M2 was selected for the study. Three samples of M2 were taken out and physicochemical tests were performed and the drug content uniformity of individual samples of sols was determined using drug content uniformity procedure. The results are given in table-4.

3. RESULTS AND DISCUSSION
All formulations were free flowing liquids, almost clear and yellow in appearance probably due to the presence of polymers and inherent color of the drug respectively. All the formulations when checked for clarity did not reveal the presence of any foreign impurities. The sols on undergoing phase transition were found to be stiff and transparent in nature. The pHs of all the sols were very close to the physiological pH while the gels were completely neutral.

3.1 Solid State Compatibility
Study indicates that the peaks in the physical mixture spectra correlate with the drug spectrum ranging from 400-4000cm^{-1}. Also, there were neither alterations in major /characteristics peaks nor the appearance of any extra peaks indicating that the drug is compatible with all the added excipients as shown in fig 1, 2, 3 and 4.

3.2 Liquid State Compatibility
Study was carried out on all the four formulations along with pure drug and the Rf values were calculated individually. Rf values depicted that, the spots of the sample were indeed of unchanged Moxifloxacin hydrochloride, as the Rf value of the Standard (pure drug) ie. 0.52 and the samples were coinciding vividly. Besides there were no other additional spots on the developed plate indicating that the drugs chemical form was not altered.

3.3 *In vitro* Studies
All the formulations were subjected to in-vitro release studies. Formulation M1 i.e. formulation with only gelling agent Carbopol 980 and without viscoliser was found to release drug for a period of 8 hours. And the cumulative percentage drug released was found to be 96.90 (Figure 5). The decrease in the duration of drug release may be attributed to weak gel form.
All the formulations were found to release drug for a period of 12 hours. The cumulative percentage drug release of formulation M2, M3 and M4 at the end of 12 hours were 98.92, 97.33 and 97.01 respectively (Figure 6) .The increase in the duration of drug released may be attributed to the presence of viscolisers which helped to increase the rigidity and integrity of the gel formed in situ. Within the formulations, formulation M2 (containing HPMC) showed greatest cumulative drug release while formulation M3 (containing HEC),and formulation M4 (containing PVA) showed almost similar drug release though formulation M4 was found to be of lowest viscosity in comparison to formulation M2 and M3. It can be seen that formulations containing individual viscolisers contributed to more complete and extended drug release in comparison to formulation containing only gelling agent.

3.4 Microbiological Studies
All formulations passed the tests for sterility as no growth was observed in the formulations at the end of 14 days. The Minimum Inhibitory Concentration of Moxifloxacin hydrochloride was found to be 4 µg/ml. Carbopol with and without the added viscolisers did not interfere with the antibacterial properties of Moxifloxacin hydrochloride when compared with marketed formulation. And formulation M2 showed good inhibitory results in comparison with other formulations and marketed formulation and proved to be the best developed formulation as it ,exhibited a proper balance between the various physical parameters namely viscosity, gelling strength, release kinetics and good antimicrobial properties.

3.5 Biological Studies
The rabbits subjected to ocular irritancy test did not show any signs of irritation, inflammation, lacrymation or abnormal discharge of any type (Figure 7).
Stability study was carried out for formulation M2 as it satisfied all the requirements of best formulation.

Table 1: Study of Combination of gelling agent with Viscoliser (HPMC)

Gelling agent	Viscoliser	Gelling Capacity	Rheology of Sol
0.3%	0.5%	+	Low viscosity
0.3%	0.7%	++	Low viscosity
0.3%	1%	++	Highly viscous
0.4%	0.5%	+++	Low viscosity
0.4%	0.7%	+++	Viscous
0.4%	1%	+++	Highly viscous
0.5%	0.5%	+++	Low viscosity
0.5%	0.7%	+++	Highly viscous
0.5%	1%	+++	Highly viscous

- No gelation
+ Gels after few minutes and dissolves rapidly
++ Gelation immediate remains for a few hours
++ Gelation immediate remains for an extended period of time

Table 2: Composition of Formulations

Ingredients	Formulation Code			
	M1	M2	M3	M4
Moxifloxacin hydrochloride	0.5 %	0.5 %	0.5 %	0.5 %
Carbopol 980	0.7 %	0.4 %	0.4 %	0.4 %
HPMC	-	0.5 %	-	-
HEC	-	-	0.5 %	-
PVA	-	-	-	0.5 %
Benzalkonium chloride	0.01%	0.01%	0.01%	0.01%
Sodium chloride	0.8%	0.8%	0.8%	0.8%
Sodium hydroxide	0.1%	0.1%	0.1%	0.1%
Phosphate buffer	q.s	q.s	q.s	q.s

Table-3: Evaluation of Formulations

Formulation Code	*pH of Sol*	*pH of Gel*	Viscosity of Sol in cps	Viscosity of Gel in cps	Gelling Capacity	Drug Content Uniformity	Cumulative % Drug released	Sterility test
M1	5.50	6.89	4000	6000	++	100 ± 0.20	96.90±0.45*	No growth
M2	6.10	7.00	6000	7500	+++	101.16 ± 0.45	98.92±0.25	No growth
M3	6.20	7.12	9500	12500	+++	98.60 ±0.30	97.33±0.22	No growth
M4	6.00	6.98	4500	6750	+++	98.34 ±0.65	97.01±0.45	No growth

- No gelation
+ Gels after few minutes and dissolves rapidly
++ Gelation immediate remains for a few hours
+++ Gelation immediate remains for an extended period of time
* Release at the end of 8th hour

Table 4: Stability study

Formulation Code	% Drug content uniformity
M2	101.70 ± 0.20

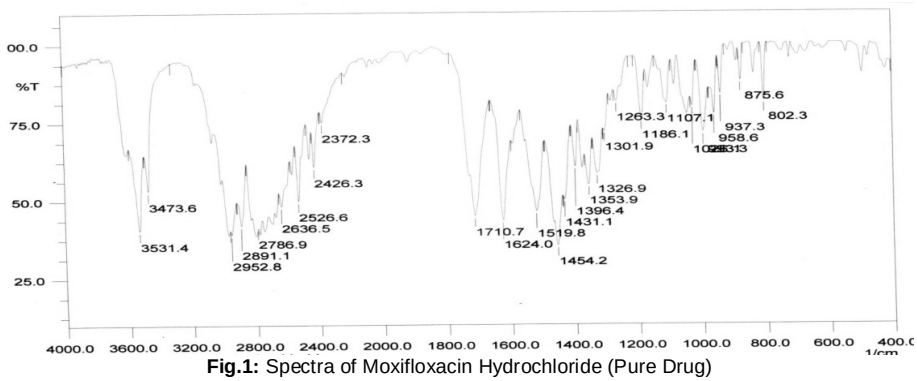


Fig.1: Spectra of Moxifloxacin Hydrochloride (Pure Drug)

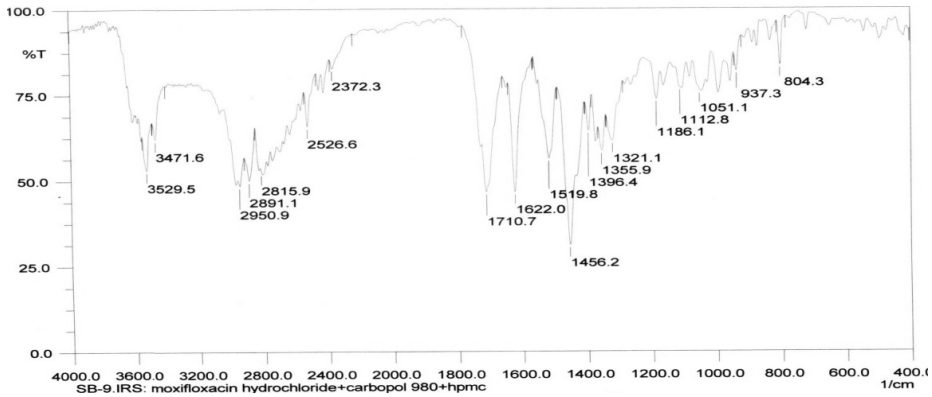


Fig.2: Spectra of Physical Mixture M2

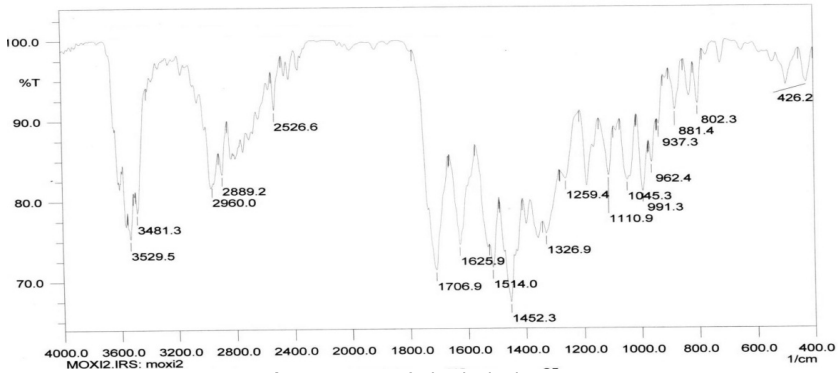


Fig.3: Spectra of Physical Mixture M3

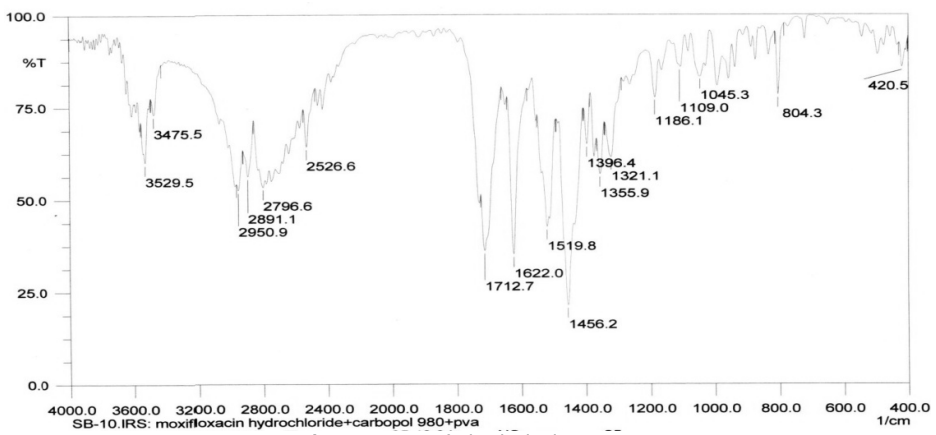


Fig.4: Spectra of Physical Mixture M4

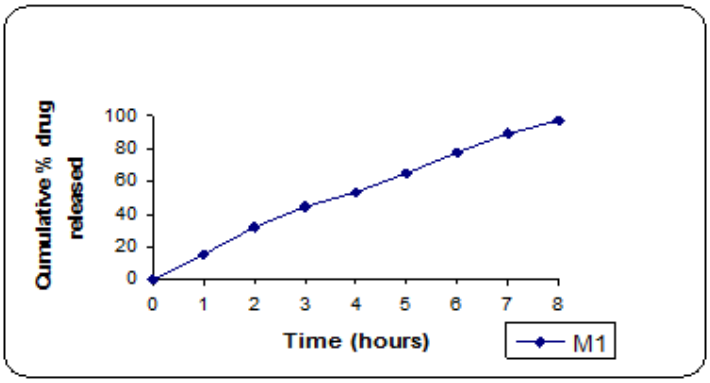


Fig.5: Zero order plot for formulation **M1**

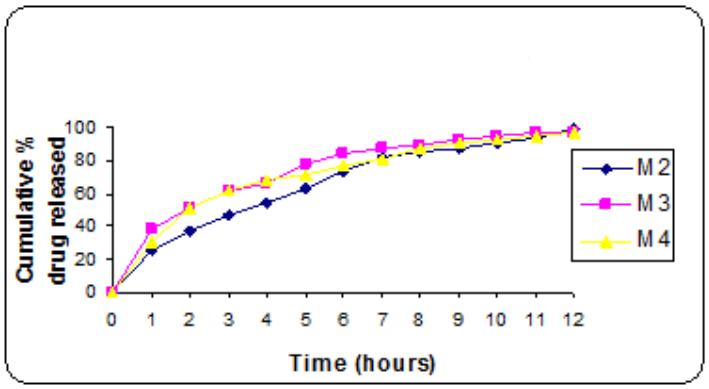
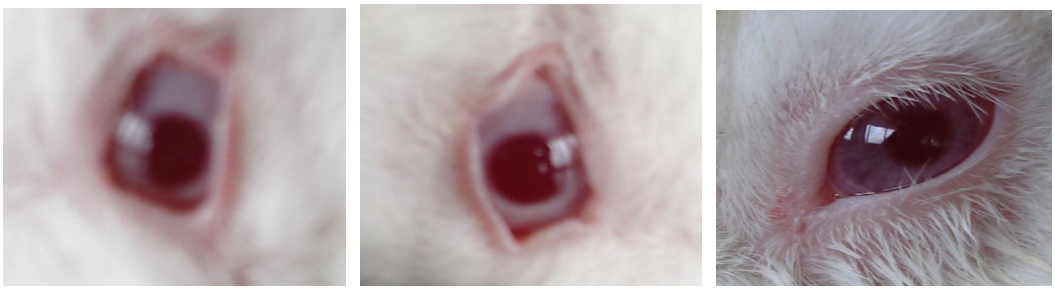


Fig. 6: Zero order plot for formulation **M2, M3** and **M4**



Right eye as control | Left eye before test | Ocular Irritancy test

Fig.7: Biological test

4. CONCLUSION

The devised formulation met the objectives and all the expected targets of this research study.The developed formulations were found to be efficacious, stable, non-irritant and thus a viable alternative to conventional eye drops by virtue of its ability to sustain drug release over a 12 hour period.

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