Research Article CODEN: IJPRNK ISSN: 2277-8713
Mitul Patel, IJPRBS, 2013; Volume 2(6): 140-155 IJPRBS

INTERNATIONAL JOURNAL OF
PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

FORMULATION AND EVALUATION OF SUSTAINED OPHTHALMIC GEL FORMING SYSTEM OF LEVOFLOXACIN

MITUL PATEL, BHAVIN BHIMANI, KETAN RANCH, DR. UPENDRA PATEL, DR. RAGIN SHAH, DR. DINESH R. SHAH[2]

1. ASPBRI, Adalaj, Ahmedabad.
2. Maliba Pharmacy College Bardoli.

Accepted Date: 20/11/2013; Published Date: 27/12/2013

Abstract: Conventional ophthalmic solutions often eliminate rapidly after administration and can't provide and maintain an adequate concentration of drug in the precorneal area. This can be overcome by the use of in *situ* gel forming systems that are instilled as drops into the eye and undergo a sol-gel transition in the cul-de-sac. The present work describes the formulation and evaluation of an ophthalmic delivery system of an antibacterial agent, levofloxacin, based on the concept of pH sensitive and temperature sensitive in *situ* gelation. The pH sensitive polymer Carbopol 940 and temperature sensitive Poloxamer 407 were used as gelling agent with HPMC E50LV which acted as viscosity enhancing agent. These combine solution would convert to form gels under physiological condition. The rheological properties and in vitro drug release test of various formulations were evaluated. In this study, 2^3 full factorial designs was used to investigate the joint influence of three formulation variables, amount of Poloxamer 407, Carbopol 940 and amount of HPMC E50LV at two level. The results of multiple linear regression analysis revealed that for obtaining desired viscosity and in vitro release pattern, eye drops should be prepared using an optimum concentration of all three polymers. The formulations showed a significant gelling in physiological condition. In vitro release study demonstrated diffusion controlled release of levofloxacin from formulations over a period of 6-8 h. The results also demonstrated that Carbopol/Poloxamer mixture can be used along with HPMC E50LV as in *situ* gelling vehicle to enhance patient compliance. The developed formulation was therapeutically efficacious, stable, non-irritant and provided sustained release of drug over a period of 6-8 h. The developed system is thus a viable alternative to conventional eye drops.

Keywords: Levofloxacin, In situ, Carbopol 940, Poloxamer 407, HPMC E50LV , 2^3 full factorial design

PAPER-QR CODE

Corresponding Author: MR. MITUL PATEL

Access Online On:

www.ijprbs.com

How to Cite This Article:

Mitul Patel, IJPRBS, 2013; Volume 2(6): 140-155

INTRODUCTION

Millions of people suffer from various ocular diseases, many of which lead to irreversible blindness. Among ocular diseases, the posterior segment ocular diseases, AMD (Age-related macular degeneration), diabetic retinopathy, and glaucoma, are the main causes of irreversible blindness in developed countries. [1] Ocular therapeutic agents can be delivered to a target site by several types of drug delivery methods. Methods of ocular drug delivery are roughly categorized into three methods, topical administration, injection, sustained-release implant.

Systemic administration of therapeutic agents has been used for the treatment of ocular diseases.[2] However, only 1-2% of plasma drug concentration reaches the vitreous cavity due to effective tear drainage, blinking action of eye so the drastic reduction of drug concentration . To achieve a therapeutic drug level in the eye, frequent systemic administration with high drug doses are required which may induce systemic side effects. [3, 4] Ocular drug delivery by topical administration such as eye drops accounts for nearly 90% of ocular therapeutic formulations. [5] Few topically applied drugs can reach the posterior segment of the eye because of the long diffusion distance and the rapid clearance by aqueous humor flow. Several preparations are available in market to increase precorneal residence time with reduce drug elimination. For that in *situ* gelling systems are widely useful.[6,7]

The use of preformed gels still has drawbacks that can limit their interest for ophthalmic drug delivery or as tear substitutes with drawbacks of blurred vision, crusting of eyelids, and lachrymation. A new approach is to try to combine advantages of both solutions and gels, such as accuracy and facility of administration of the former and prolonged residence time of the later. [8] Thus in *situ* gels can be instilled as eye drops and undergo an immediate gelation when in contact with the eye. In *situ*-forming gels are liquid upon instillation and undergo phase transition in the ocular cul-de-sac to form viscoelastic gel and this provides a response to environmental changes. [9]

A new approach is to try to combine advantages of both solutions and gels, such as accuracy and facility of administration of the former and prolonged residence time of the later. Here Levofloxacin which is antibiotics used for eye infection. Topical ophthalmic dose of Levofloxacin is 1-2 drops of 0.5 % w/v solution in affected eye every 4 h and hourly in the case of severe infections. Eye infection treatment needs the drug residence for longer duration in eye to treat inflammation, so that the damage on optic nerve will be less.

The concept to be investigated involves the development of Sustained gel forming Ophthalmic Drug Delivery System which will deliver the fraction of drug for longer duration with increased corneal residence time and improved bioavailability of the drug.

The present study aims at developing a sustained ocular drug delivery system of Levofloxacin based on concept of gel forming systems, to treat external infection of eye such as acute and sub acute conjunctivitis, bacterial infection and kara to conjunctivitis. In that Carbopol 940,Poloxamer 407and HPMC is used as polymeric system. Poloxamer having pH dependent gelling ability.

The idea behind this work is that, an aqueous composition reversibly converts into gel & response to simultaneous change in at least two physical parameters such as pH and temperature, can be formed by using combination of polymers which exhibits reversible gelation property. This gel forming system can be formed by using polymers which exhibits reversible gelation property with change in pH, temperature and ionic strength.

MATERIALS AND METHODS

Material used for study

Levofloxacin was obtained from Mepro Pharmaceuticals Pvt. Ltd., Wadhvan, India. Poloxamer 407 was obtained for BASF Corporation, New York, USA. Hydroxy propyl methyl cellulose E50LV was obtained from DOW Chemicals. Carbopol 940 from Corel Pharma. Boric acid , Benzalkonium Chloride, Sodium chloride, Sodium bicarbonate ,Calcium chloride was obtained from S. D. Fine Chemicals Ltd.

Preparation of in *situ* gelling system

Preparation of formulation

Aqueous solution of different concentration of pH sensitive carbopol 940, thermo sensitive Poloxamer 407 and viscosity enhancing agent HPMC E50LV (formulation codes F1 to F8) were prepared in citro phosphate buffer pH 5.0.

The buffer salts were dissolved in double distilled deionized water, HPMC E50LV (0.4% w/v and 0.8 % w/v) was dissolved in it. This HPMC E50 LV solution prepared in phosphate buffer pH 5 was used to incorporate carbopol 940 and poloxamer 407. The carbopol solutions were prepared by dispersing the required amount in the above prepared HPMC E50LV solutions with continuous stirring until completely dissolved. For preparation of poloxamer 407 solutions, the required amount of poloxamer 407 was dispersed in above prepared HPMC E50LV solutions with continuous stirring for 1 h. The partially dissolved poloxamer solutions were stored in the refrigerator until the entire polymer was completely dissolved (approximately 24 h). The carbopol/poloxamer solutions were prepared by dispersing the required amount of poloxamer in the desired concentration of carbopol with continuous stirring for 1 h.

The partially dissolved solutions were refrigerated until solutions were thoroughly mixed (approximately 24 h). The reported composition of carbopol/poloxamer mixture was the final concentration of carbopol and poloxamer content in the mixture as given in the table 4.6

The required quantity of levofloxacine and benzalchonium chloride was dissolved in the citro phosphate buffer pH 5.0. This solution was added to the carbopol/poloxamer/HPMC solution under constant stirring until uniform and clear solution was obtained. Citro phosphate buffer pH 5 was added to make up the volume 100 ml. The developed formulations were filled in 5-ml capacity amber glass vials, closed with gray butyl rubber closures and sealed with aluminum caps. The formulations, in their final pack, were subjected to terminal sterilization by autoclaving at 121^{0C} and 15 psi for 20 min. Boric acid was used as an isotonicity adjusting agent and its quantity in formula was calculated by sodium chloride equivalent method. Formula is given in table 4.

Optimization Using Factorial Design:

In this study, a 2^3 factorial plan was used to determine the effects of formulation variables. In this design three factors are evaluated, each at two levels, and experimental trials are performed at all eight possible combinations. The amount of poloxamer 407(*X1*), amount of carbopol 940 *(X2)* and amount of HPMC E50LV (X3) was selected as independent variables. The viscosity at 12 RPM (physiological condition) and t90% were selected as dependent variables. The three factors and their levels are shown in Table 1.

The results obtained are shown in Table 2 and 3 . For the Viscosity at 12 RPM (physiological condition), all three factors were found to be statistically significant ($P<0.05$): level of poloxamer 407, Carbopol 940 & HPMC E50LV and the interactions between these factors were also statistically significant. For t90% level of all three factors were found to be statistically significant ($P<0.05$): level of poloxamer 407, Carbopol 940 & HPMC E 50LV and interactions among all factors were also statistically significant.

Evaluation parameter:

Drug content[10]

Levofloxacin (0.5% w/v) containing preparation were shaken for few minute and prepared 100 µg/L stock solution of final preparation using artificial tear fluid pH 7.4. From that 10 µg/L solution was prepared and measure at 287 nm. The results obtained are as shown in table 5.

Clarity [11]

The clarity of the formulation after and before gelling was determined by visual examination of the formulation under light alternatively against white and black backgrounds.

In vitro Gelling Capacity [12, 13]

The gelling capacity was determined by freshly prepared drop of system in a vial containing 2ml of freshly prepared artificial tear fluid (pH 7.4) and equilibrated at 37ºC. The visual assessment of gel formation was carried out. Time required for gelation as well as time taken for the formed gel to dissolve were also noted. Different grades were allotted as the gel integrity, weight, and rate of formation of gel with respect to time. The results obtained are as shown in table 5.

Viscosity [13]

The prepared formulations were evaluated for viscosity in order to identify the compositions that best suit for use as in *situ* gelling systems. The viscosity of the systems was measured using Brookfield viscometer (LV DVII +PRO model) at 12 rpm for the purpose of comparative evaluation at non physiological condition. The results obtained are as shown in table 5.

Rheological profile [14]

The rheological studies of samples were carried out with Brookfield Viscometer (LV DVII +PRO model). The formulations (pH 5.0, RT 25 0C) were poured into sample adapter of the Brookfield Viscometer and angular velocity was increased gradually from 0.3 to 30 RPM. The hierarchy of the angular velocity was reversed. The average of the two readings was used to calculate the viscosity. The formulation was then poured into an ointment jar and the pH raised to 7.4 (37 0C) by adding freshly prepared ATF. The viscosity measured at both the pH for individual formulation is plotted against angular velocity (RPM). Result shown in figure 1.

In-vitro drug release study [15,16]

The In-vitro release studies of levofloxacin from the formulation were studied through the cellophane membrane using a modified USP XXIII dissolution apparatus. The dissolution medium used was freshly prepared artificial tear fluid (pH 7.4). A cellophane membrane previously soaked overnight in the dissolution medium was tied to one end of the specifically designed glass cylinder (open at both end of 5 cm diameter). One ml volume of the formulation (equivalent to 5 mg) was accurately pipetted into this assembly. The glass cylinder was suspended in 100 ml of dissolution medium at 37±0.5 ºC, so that the membrane just touches the receptor medium surface. A Teflon TM coated magnetic bar continuously stirred the

receiving medium at 50 rpm to avoid diffusion layer effects. A sample was placed evenly on the surface of the membrane in the donor compartment. Aliquots, each of 5 ml volume were withdrawn at hourly interval and replaced by an equal volume of dissolution medium to maintain the sink condition. The aliquots were diluted with dissolution medium and analysed by UV Spectrophotometer at 287 nm. The results obtained are as shown in figure 2.

Other evaluation test such as antimicrobial efficacy studies, effect of sterilization, eye irritation test and stability were carried out only on optimized and selected F4 batch.

Antimicrobial efficacy studies [15]

Antimicrobial efficacy was determined by agar diffusion test employing cup plate technique. The microbiological studies ascertained the biological activity of the optimised formulation F4 and marketed eye drops against microorganism *Pseudomonas arugenosa* and *Staphyllococcus aurious* as test microorganism. A layer of nutrient agar (20 ml) seeded with the test micro organism was allowed to solidify in Petri dish. Cups were made on the solidified agar layer with the help of sterile borer with 4 mm diameter. Marketed sterile formulation and developed formulation diluted suitably to 5 and 50 µg/ml solution and were poured into cups of agar plates. After allowing diffusion of solution for two hours, the agar plates were incubated at 37ºC for 2 hrs. The zone of inhibition (ZOI) was measured around each cup and was compared with the marketed formulation. The entire operation except the incubation was carried out in a laminar air flow unit. Each solution was tested in triplicate. The results obtained are as shown in table 7.

Eye irritation test [16]

The potential ocular irritancy and/or damaging effects of the formulations under test were evaluated by observing them for any redness, inflammation or increased tear production. Selected formulation F4 was tested on three rabbits; the treatment was performed by a single instillation (50µl) of the formulation under test into the conjunctival sac of one eye. Both eyes of the rabbits under test were examined for any signs of irritation before treatment and 30 min 60 min, 120 min, and 180 min after instillation of 50µl of selected formulation. The results obtained are as shown in table 8.

Stability Studies

The selected sterile formulation (F4) was stored at 40 0C for 3 months and formulation was finally evaluated for drug content, viscosity, pH and in vitro drug release. The formulation was found to be clear after 3 months of storage and no change in drug content, pH, viscosity and in vitro drug release.

RESULTS AND DISCUSSION

Clarity, pH and drug content

Clarity of the formulations was found to be satisfactory. The formulations were light yellow in color. Terminal sterilization with autoclaving had no effect on the physicochemical properties of the formulations. The haziness was observed after autoclaving. The haziness was due to precipitation of HPMC E50LV at elevated temperature. Haziness was found to disappear and the original clarity was regained after overnight standing. The pH was within acceptable range and hence would not cause any irritation upon administration of the formulation. Table 5 shows the result of percent drug content for all the formulations. The drug content was found to be in acceptable range for all the formulations. Percent drug content in all four formulation were in the range 98-102 % indicating uniform distribution of drug.

Table 5. Drug content, pH, and in vitro gelling Capacity & viscosity of F1 to F8 formulations.

In vitro Gelling Capacity and viscosity

Different combination of Poloxamer 407, HPMC E50LV and Carbopol 940 were tested for gelling capacity. Gelling capacity was evaluated on visual basis against white and black back ground on increasing the pH and temperature. At relatively lower concentration the rates of gelation were low, hence not suitable for ophthalmic formulation, as they might be removed from the cul-de-sac by the tear and blinking before gelation occurs. The formation of a strong gel is contigent on a short gelation time of the delivery system upon instillation into the eye and polymer concentration in combination (Poloxamer 407 10%, HPMC E50LV 0.8% and Carbopol 940 0.4%) fulfill this criterion. Thus such vehicles will not be susceptible to drainage from the eye as seen in the case of conventional ophthalmic solutions.

Figure1: Comparative viscosity data of formulations (F1-F8) and marketed eye drops in physiological conditions.

In viro Drug release Study

All the formulations were evaluated for drug release pattern. The drug release profile

of marketed eye drops (Levoflox, conventional eye drops) was compared with the prepared formulations. The data and curves obtained from in vitro release test are as follows.

Figure 2: Comparative drug release profile of in situ gelling system of F1-F8 formulation with marketed formulation

Release kinetic profile

To gain a better insight into the mechanisms underlying the release of levofloxacin from in situ gel forming system and their role in ophthalmic delivery of levofloxacin, the release kinetics of levofloxacin was investigated. Dissolutuion data was given zero order, first order, Higuchi and Korsmeyer kinetic treatment for all the formulations (table 6). These different kinetic equations were applied to interpret the release rate

from all the formulations. The best with higher correlation coefficient (R2 < 0.98) was

found with Higu indicating that release from gel forming system is based on diffusion mechanism for F3, F4, F5 and F6. The value of release exponent is more than 0.45 and less than 0.89 indicating non-fickian anamolous release from F1, F4, F6, F7 and F8.

Table 6: release kinetic profile

Effect of sterilization [15]

The autoclaving exerted non significant effect on drug content, viscosity and pH of the formulations. However, haziness was observed in formulation coded as F5, F6, F7 and F8 after autoclaving due to precipitation of HPMC E50LV at elevated temperature. But it was found that haziness disappeared and original carity was regained after overnight storage at ambient condition.

Table 7: Effect of sterilization

Eye irritation test [16]

Selected formulation F4 did not showed any redness, inflammation or increased tear production in rabbits. The result of ocular irritation studies indicates that all formulations are non irritant. Excellent ocular tolerance was noted. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae were visible.

Table 8: Eye irritation test

Levofloxacin a broad spectrum antibacterial agent used in the treatment of ocular infections was successfully formulated as in *situ* gel forming eye drops (0.5% w/v) using poloxamer 407 and carbopol 940 as gelling agents and HPMC E50 LV as a viscosity enhancing agent. The formulation was liquid at the formulated pH 5 and gel formed at physiological pH 7.4 The gel formed at in *situ* afforded sustained drug release over 6-8 h periods. Stability data recorded over a 3 month period under elevated temperature conditions indicated the formulation to be stable. The developed formulation is a viable alternative to conventional eye drop by virtue of it to sustain drug release. It is also observed that when pH sensitive and temperature sensitive polymers are used along with HPMC E-50LV, the effect due to HPMC E50LV was statistically

significant on viscosity. Viscosity (Physiological condition) of Batch F4 was found to be much similar with the marketed gel forming dosage form Timolate .It is thus concluded that by adopting a systematic formulation approach, an optimum point can be reached in the shortest time with minimum efforts to achieve desirable rheological and in vitro release property for in *situ* gel forming system. Therefore, the combined approach can be used as in *situ* gelling vehicle for ophthalmic drug delivery.

Acknowledgement

The authors thanks to corel pharma, BASF corporation and dow chemicals for provide gift sample of chemicals required for research work. Authors also thankful to Mepro Pharmaceuticals for providing Levofloxacin as a gift sample.

Table 1: Full factorial design layout for 2^3

Batch code	X1	X2	X3
F1	-1	-1	-1
F2	1	-1	-1
F3	-1	1	-1
F4	1	1	-1
F5	-1	-1	1
F6	1	-1	1
F7	-1	1	1
F8	1	1	1

X1 - Poloxamer 407

X2 - Carbopol 940

X3 - HPMC E50LV

Table 2: Coded value for Poloxamer 407, Carbopol 940 and HPMC E50LV

Coded value			
Coded Value	**Poloxamer 407 (%w/v) X1**	**Carbopol 940 (%w/v) X2**	**HPMC E50LV (%w/v) X3**
-1	5	0.2	0.4
+1	10	0.4	0.8

Table 3: Optimization using check point batch

Batch code	Poloxamer 407	carbopol 940	HPMC E50 LV	Viscosity at 12 rpm	t90%(min)
F1	-1	-1	-1	20900	149.1698
F2	1	-1	-1	40900	265.6848
F3	-1	1	-1	38400	249.7729
F4	1	1	-1	40900	345.7812
F5	-1	-1	1	40000	155.2458
F6	1	-1	1	60000	231.3585
F7	-1	1	1	73000	348.4132
F8	1	1	1	67000	362.5869
Check point	+2(8%)	-0.5 (0.25 %)	+1 (0.8%)	56351.76 (55700)	239.823 (249.30)

Table 4: Formula of F1 to F8 formulations

Ingredients	Formulation batch code							
	F1	F2	F3	F4	F5	F6	F7	F8
Levofloxacin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Poloxamer 407	5	10	5	10	5	10	5	10
Carbopol 940	0.2	0.2	0.4	0.4	0.2	0.2	0.4	0.4
HPMC E50LV	0.4	0.4	0.4	0.4	0.8	0.8	0.8	0.8
Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Boric acid	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.64
Citro phosphate buffer pH 5(q.s.)	100	100	100	100	100	100	100	100

Note: all units are in % w/v

Table 5. Drug content, pH, and in vitro gelling Capacity & viscosity of F1 to F8 formulations.

Batch	pH	Drug content	Gelling capacity	Viscosity in cps At 12 rpm
F1	5	98.23	-	114
F2	5.4	98.32	++	170
F3	5.2	99.16	++	110
F4	5.3	101.12	+++	120
F5	5	99.56	-	1720
F6	5.6	100.34	++	1820
F7	5.2	101.66	++	1220
F8	5.4	99.63	+++	1467

- No gelation

+ Gelation after few minutes & remain few hr

++ Gelation immediate & remain few hr

+++ Gelation immediate & remain extended time

Table 6: Release kinetic data for F1-F8 preparation.

	Correlation coefficient			Release Exponent
Formulation Code	Zero order R^2	First order R^2	Higuchi R^2	Korsmeyer 'n'
F1	0.5539	0.6635	0.7609	0.7606
F2	0.8995	0.9474	0.9566	0.4389
F3	0.8877	0.9439	0.9858	0.378
F4	0.7861	0.9138	0.9806	0.4972
F5	0.8319	0.9369	0.9896	0.2933
F6	0.802	0.9327	0.9897	0.556
F7	0.9468	0.9613	0.9513	0.5025
F8	0.7627	0.7934	0.8863	0.5291

Table 7: Effect of sterilization

Concentration(µg/ml)	Std ZOI(cm)	test ZOI(cm)	Percent efficiency
Staphyllococus aureous			
5	1.5	1.4	93.33
5	1.6	1.6	100
50	2.6	2.5	96.15
50	2.9	2.8	96.55
Pseudomonas aeruginosa			
5	1.1	0.9	81.81
5	1.1	1	90.9
50	1.9	1.6	84.21
50	1.8	1.5	88.33

Table 8: Eye irritation test

Preparation	Avarage score
Marketed Formulation	0
Blank of F6	0
F6	0

0: None

1: Intermediate itching with slightly watering and eye become pink

2: Mild itching with occasional need to wipe eye and more intense redness of eye

3: Severe itching with tears rolling down cheeck and deep red colouration of eye

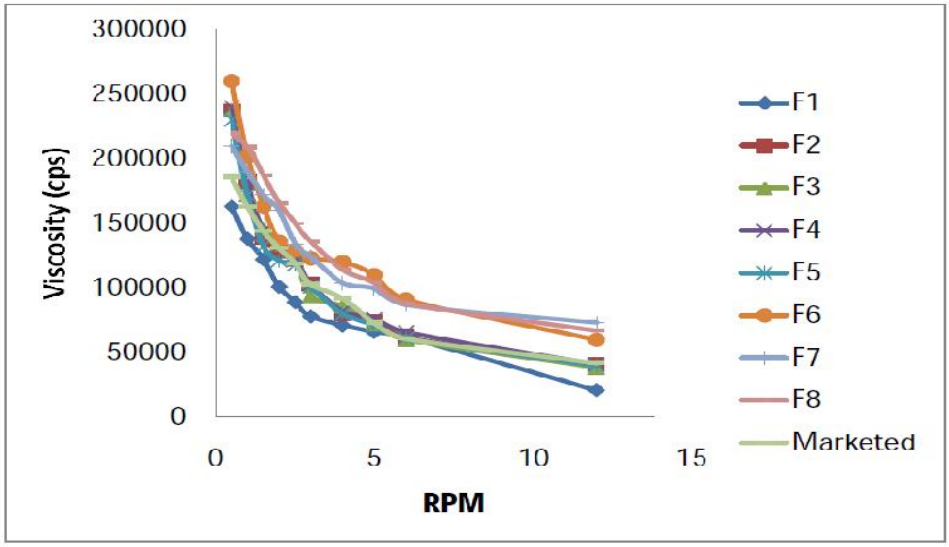

Figure1: Comparative viscosity data of formulations (F1-F8) and marketed eye drops in physiological conditions.

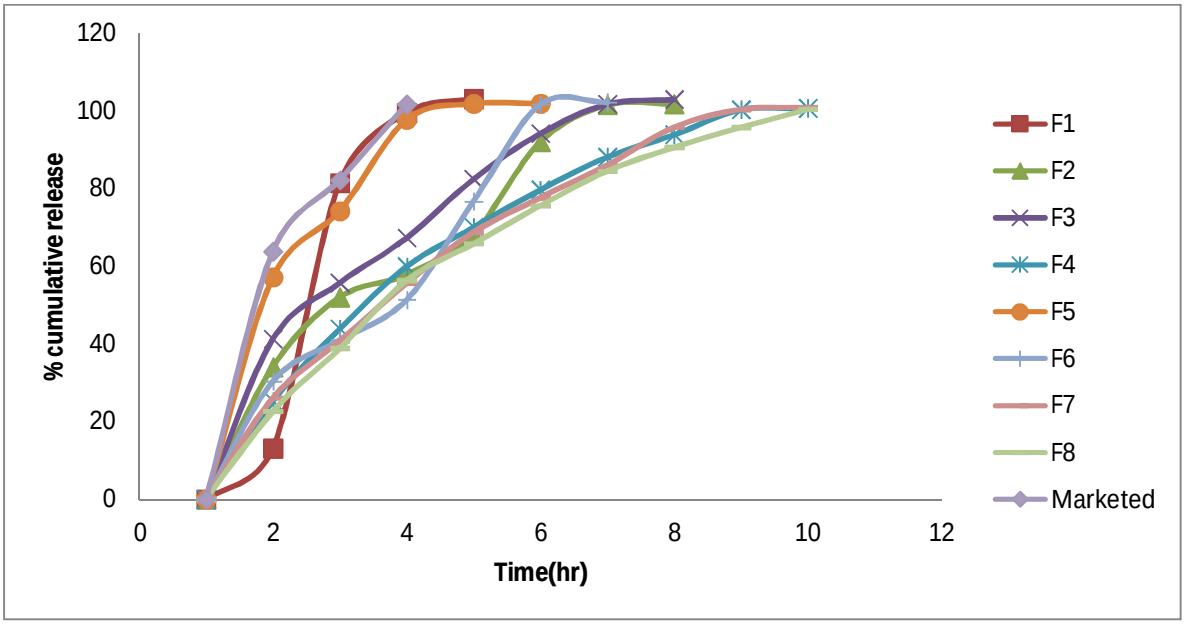

Figure 2: Comparative drug release profile of in situ gelling system of F1-F8 formulation with marketed formulation

References

1. Clark AF, Yorio T. Ophthalmic Drug Discovery. Nat Rev Drug Discovery 2003; 2(6): 448-59.

2. Geroski DH, Edelhauser HF. Drug delivery for posterial eye disease. Invest Ophthalmol Vis Sci 2000;41(5): 961-4.

3. Jun Mok Lee, MD, Woo Ho Nam, MD, and Ha KyoungKim. Photodynamic Therapy With Verteporfin for Choroidal Neovascularization in Patients with Angioid Streaks.MD Department of Ophthalmology, 2003. 110(5): p. 979-86.

4. Gillies MC, Simpson JM, Luo W, Penfold P, Hunyor AB, Chua W, Mitchell P, Billson F. A randomized clinical trial of a single dose of intravitreal triamcinolone acetonide for neovascular age-related macular degeneration: oneyear results. Arch Ophthalmol 2003;121(5): 667-73.

5. Jonas JB, Kreissig I, Sofker A, Degenring RF. Intravitreal Injection of Triamcinolone for Diffuse Diabetic Macular Edema. Arch Ophthalmol2003;121(1): 57-61.

6. Musch DC, Martin DF, Gordon JF, Davis MD, Kuppermann BD. Treatment of cytomegalovirus retinitis with a sustained-release ganciclovir implant. The Ganciclovir Implant Study Group.N Engl J Med 1997;337(2): 83-90.

7. Kurz D, CiullaTA. Novel approaches for retinal drug delivery. OphthalmolClin North Am 2002;15(3): 405-410.

8. Kumar S, Haglund BO, Himmelstein KJ. Ophthalmic drug delivery systems. J. Ocul. Pharmacol.1994;10 (1) :47-56.

9. Miller SC, Donovan MD. Effect of poloxamer 407 gel on the miotic activity of pilocarpine nitrate on rabbits. Int. J. Pharm. 1982;12:147-152.

10. Pandit JK, Bharthi D, Srinatha A, Ridhurkar DN, Singh S. Longacting ophthalmic formulation of Indomethacin: evaluation of alginate gel systems. Indian J Pharm Sci. 2007;69:37-40

11. Gupta H, Sharma A, Srivastava B. Pluronic and chitosan based in situ gel system for periodontal applications. Asian J Pharma. 2009:94-6

12. Miyazaki S, Suzuki S, Kawasaki N, Endo K, Takahashi A, Attwood D.In situ gelling xyloglucan formulations for sustained release ocular delivery of pilocarpine hydrochloride, Int. J. Pharm.2001;229: 29-36.

13. Rozier A, Mazuel C, Grove J, Plazonnet. Gelrite®: a novel ionactivated in situ gelling polymer for ophthalmic vehicles. Effect on bioavailability of timolol, Int. J. Pharm.1989;57: 163-168.

14. Lin HR, Sung KC. Carbopol pluronic phase change solution for ophthalmic drug delivery. J Contr Rel. 2000;69:378-388

15. Edsman K, Carlfors J, Petersson R. Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. Eur J Pharma. 1998;105-112

16. Ganguly S, Dash AK. A novel in situ gel for sustained drug delivery and targeting. Int J Pharma. 2004; 276: 83-92.