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Design and Characterization of pH Triggered *In-Situ* Gel Containing Moxifloxacin for Ophthalmic Delivery.

Nagesh C*, Patel Sujit, Suma Naduvinamani, and Chandrashekhara S.

Department of Pharmaceutics, Maratha Mandal's College of Pharmacy, Belgaum - 590016, Central Research Laboratory, MM Group of Institutes, Belgaum, Karnataka, India.

ABSTRACT

The aim of present investigation was to develop pH triggered *in situ* ophthalmic gel containing moxifloxacin. The *in situ* gel was prepared using Polyox 301 - a pH sensitive gelling agent to improve duration of localization of the preparation with cornea. These systems are prepared as eye drops; they undergo reversible phase transformation (sol to gel) within the cul-de-sac as the preparation attained to a required pH. The gel increases the contact time, thereby increases the ocular bioavailability and reduce the administration frequency. The other components present in the formulation are hydroxyl propyl methyl cellulose K_4M as viscosity imparting agent, sodium chloride as a tonicity modifier and benzalkonium chloride as a preservative. The prepared formulations were evaluated for appearance, pH, drug content uniformity, *in vitro* gelation studies, rheological studies, and test for sterility, *in vitro* release studies and stability studies. Formulation PL_4 was choosen as best formulation on the basis of its capacity to prolong the drug release till 8 hours with highest percentage of drug content i.e. 96.71±0.386 %. The viscosity before gelation was 18.5±2.75 cps and after gelation was 690.0±10.0 cps at 20 rpm. The cumulative drug release was 82.78±1.85 % after 8 hours for PL_4 formulation. These formulations showed pseudoplastic flow behavior. The results of sterility test confirmed that all the formulations were sterile. The formulations were found to be stable in stability studies.

Keywords: *in situ* ophthalmic gel, moxifloxacin, Polyox 301, pH sensitive gelling agent.

Corresponding author

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INTRODUCTION

The eye is considered as a very responsive organ of the body. Foreign materials from the eye is removed by tear flow and blinking reflex. These protective properties lead to an effective drainage of the drug when introduced into the eye [1]. Conventional ophthalmic delivery systems i.e. eye drops often results in poor bioavailability and therapeutic response due to rapid elimination of the drug from the ophthalmic cavity, drug loss, tear fluid drainage, blinking action [2]. Because of these demerits very small amount (1–6%) actually reaches in intraocular tissues, and the limited corneal permeability. To maintain drug concentration frequent instillation is required which is a situation associated with undesirable side effects caused by the systemic drug exposure. Addition of more quantity of drug in the formulation is an attempt to increase the bioavailability of the drug [3-4].

Many classes of drugs like anti-infective, anti-inflammatory agents and autonomic agents to reduce the infections caused due to various types of bacteria, fungi, viruses and also to relieve intraocular tension in glaucoma can be formulated as conventional and novel ophthalmic drug delivery systems. The novel type of ophthalmic drug delivery systems such as ocuserts, ointments, suspensions, *in situ* gels etc. have been developed to increase the ocular contact time and enhance the ophthalmic bioavailability. Now-a-days *in situ* gelling systems have been found to be favorable dosage form because of the increased contact time of drugs with corneal tissues, which results in increased bioavailability. These dosage forms are prepared using different types of polymers, which helps in the conversion of solution to gel due to change in specific physical and chemical conditions (pH, temperature, ions) in their environment; the ophthalmic cavity in this case. Basically three methods are used to prepare *in situ* gels, these are: a) pH triggered systems (e.g. poly acrylic acid, cellulose acetate hydrogen phthalate latex, pseudolatexes), b) Temperature sensitive systems (e.g. pluronics, cellulose derivatives, xyloglucans) and c) Ion-activated systems (e.g. alginates, gallen gum, carrageenan). In the present study the method selected to prepare *in situ* gel was pH sensitive gelation method using commonly used polymer i.e. Polyox 301, an environment-sensitive polymer. These preparations are able to increase or decrease its volume due to change in the pH of the environment [5].

The pH-sensitive polymers contain acidic or basic groups that either accept or release protons in response to changes in environmental pH. The polymers with a large number of ionisable groups are known as polyelectrolyte. Swelling of hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups [6-7].

By considering the above facts the present investigation was planned to develop and characterize pH sensitive *in situ* ophthalmic gel of moxifloxacin. Moxifloxacin, a broad spectrum antibacterial agent acts against gram-negative and anaerobic bacteria responsible for ocular infections. It acts by inhibiting the synthesis of enzyme DNA topo-isomerases and DNA-gyrase, which intern inhibits DNA synthesis [5, 8].

Melting point of moxifloxacin is 238-242°C; it is soluble in water, ethanol, acetone and 2-proponolol. It has a biological half-life of 12 hours. Moxifloxacin is well absorbed in gastro-intestinal (GI) tract with high volumes of distribution and penetrate intracellularly. Approximately 52% of oral or intravenous dose is metabolized via glucouronide and sulphate conjugation [8-9].

MATERIALS

Moxifloxacin was obtained as a gift sample from Centaur Pharma Pvt. Ltd., Goa, India. Polyox 301 was obtained from Colorcon Goa, India. Other chemicals and reagents used in the study were of AR grade. Equipment used in the study are UV-Visible spectrophotometer (Shimadzu Corporation, Japan), IR-spectrophotometer (Shimadzu Corporation, Japan), Brookfield viscometer (Brookfield Engineering Inc., USA), locally fabricated diffusion cells, hot plate with magnetic stirrer (Remi Equipment, Mumbai), stability chambers (Thermo labs, Mumbai), melting point apparatus (Remi Equipments, Mumbai), etc.

METHODS

Preformulation studies: Preformulation testing is the first step in development of dosage forms. Following tests were performed to identify and to know the purity and compatibility of the drug and non-drug components used in the formulations.

- **Determination of melting point:** Melting point of moxifloxacin was determined by melting point test apparatus.
- **Compatibility studies:** To check the drug-polymer compatibility, Fourier transform infrared spectrometer (IR spectrophotometer) was used [8]. The IR spectra are represented in the Figure 1 and 2.

Preparation of Polyox 301 in situ gel: Different formulations of Polyox 301 *in situ* hydrogel were prepared as per the Table 1. Required amount of HPMC K_4M (0.5 % w/v) as viscosifying agent, was first added to 70 ml of citrophosphate buffer of pH 6.0 and allowed to hydrate. Then Polyox 301 was sprinkled over this solution and allowed to hydrate overnight. 0.5 % w/v moxifloxacin was dissolved in 20 ml of citrophosphate buffer solution separately to which polymer solution was added with constant stirring until a uniform solution was obtained. Then 1 % sodium chloride and 0.02 % benzalkonium chloride were added to the formulation. Citrophosphate buffer (pH 6.0) was then added to make the volume up to 100 ml [8-10].

Table 1: Composition of Polyox 301 formulations

Sl. No.	Formulation code	Moxifloxacin (% w/v)	Polyox 301 (% w/v)	HPMC K_4M (% w/v)	Sodium chloride (% w/v)	Benzalkonium chloride (% w/v)
1.	PL_1	0.5	0.5	0.5	1.0	0.02
2.	PL_2	0.5	0.8	0.5	1.0	0.02
3.	PL_3	0.5	1.0	0.5	1.0	0.02
4.	PL_4	0.5	1.2	0.5	1.0	0.02
5.	PL_5	0.5	1.5	0.5	1.0	0.02

Acetate buffer of pH 6.5 - Quantity sufficient to produce 100 ml

EVALUATION OF *IN SITU* GEL

All *in situ* gel formulations were subjected to following evaluations.

- **Appearance:** prepared formulations were evaluated for clarity by visual observation in presence of highly illuminated light against a black and white background [5, 8 & 11].
- **pH:** The pH of the developed formulations was determined using digital pH meter [12].
- **Drug content:** One ml of the preparation was diluted to 100 ml with phosphate buffer of pH 7.4 phosphate buffer solution. From the above solution 1 ml was withdrawn and diluted to 10 ml with phosphate buffer of pH 7.4. Concentration of moxifloxacin in all formulations was determined at 296nm using UV spectrophotometer [12-14].
- **In vitro gelation studies:** The gelling capacity was determined by taking one drop of the preparation in a test tube containing 2 ml of freshly prepared simulated tear fluid (STF). Temperature was maintained at 37 ^{0} C and time taken to form gel and the gel to get dissolved was noted [5, 8].
- **Rheological studies:** The study was performed using Brookfield viscometer. Angular velocity was increased gradually from 0.5 to 50 rpm using spindle No. 62. The hierarchy of angular velocity was reversed and the average dial readings were considered to calculate the viscosity. Then the prepared solutions were allowed to gel in the STF and then again the viscosity determination was carried out. The temperature was maintained within 37±0.1 °C [5, 8].
- **Test for sterility*:*** The sterility test was performed as per Indian Pharmacopoeia. The method involves the removal of 2 ml sample from the test container with a sterile pipette or with a sterile syringe. The test liquid was aseptically transferred to fluid-thioglycolate medium (20 ml) and soyabean-casein digest medium (20 ml) separately. The liquid was mixed with the media. The inoculated media were incubated for not less than 14 days at 30 °C to 35 °C in the case of fluid-thioglycolate medium and 14 days at 20 °C to 25 °C in the case of soyabean-casein digest media [15-16].
- **In vitro drug release studies*:*** The solution to be tested (1 ml) was transferred to donor compartment. This compartment was placed over lower compartment. In between donor and receptor compartment dialysis membrane (HIMEDIA Dialysis membrane-70) was fixed. Temperature was maintained at 37±0.1°C and other conditions such as rotation of bead at 50 rpm was maintained. At regular intervals of time, 1 ml of

aliquot were withdrawn, suitably diluted and amount of moxifloxacin present each time interval was calculated by using UV spectrophotometer at 296 nm [16-17].

- **Stabilities studies*:*** The best formulation was subjected to stability studies at humidity condition at 75±5%, ambient temperature 40±2°C for a period of three months. The samples were collected at periodic interval of 0 days, 30 days, 60 days and 90 days and were evaluated for appearance, content uniformity and *in vitro* drug release studies [17,18 19].

RESULTS AND DISCUSSION

The characterization studies on the properties of *in situ* gels have been performed to investigate whether the in situ gels would be advantageous to the conventional ophthalmic drops. The *in situ* gel was prepared by varying concentration of using pH sensitive polymer i.e. Polyox 301. All the preparations were characterized for various evaluation tests.

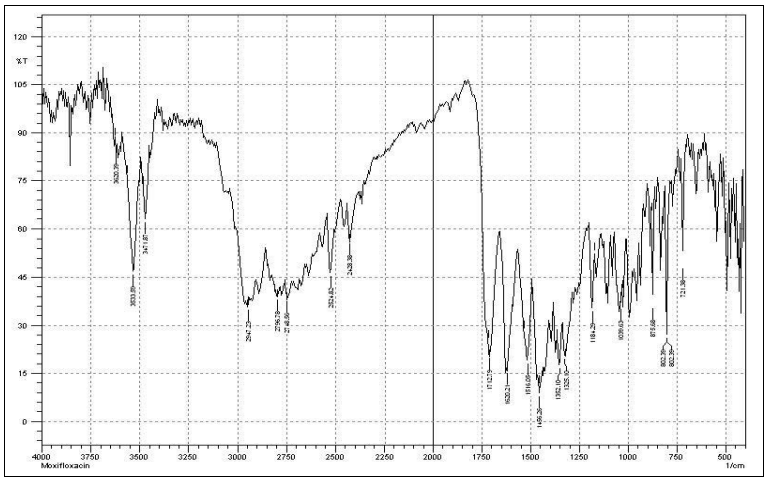

Figure 1: FTIR spectra of moxifloxacin

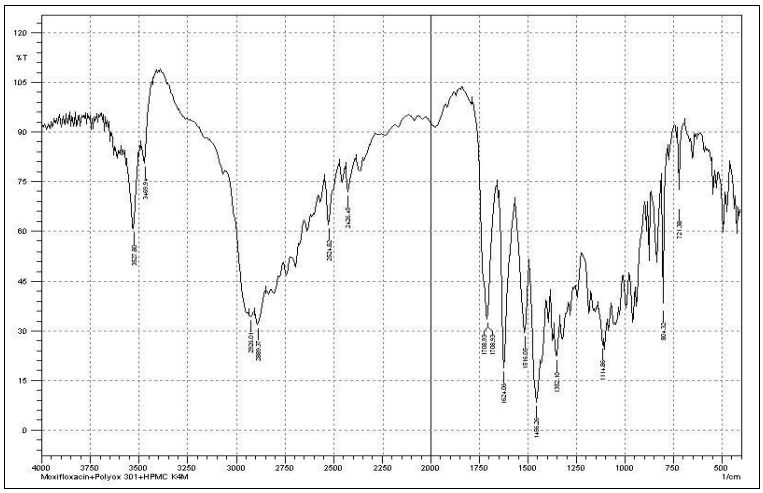

Figure 2: FTIR spectra of moxifloxacin, PL-301 and HPMC K_4M

Melting point of moxifloxacin was found to be 239^{o}C. IR peaks of moxifloxacin were observed to be 1039, 1712, 3471 and 3533 respectively for different functional groups such as C-F, C=O, N-H and –OH. These results indicated that the drug sample received was pure, as the observed frequencies are within the range of reported one. The results of compatibility studies indicated that there was no interaction between contents of the formulation. IR spectra are shown in Figure 1 & 2.

- **Appearance*:*** Clarity of all the formulations was found to be satisfactory. Terminal sterilization had left no effect on clarity of the formulations.

- **pH:** The pH of all formulations was found to be satisfactory and lies in the range 6.36 to 6.41. Terminal sterilization had left no effect on the pH.
- **Drug content:** The drug content uniformity data has shown that all the formulations were found to be uniform in drug content in the range of 96.15-97.05%.
- **In vitro gelation studies*:*** Gelation study was performed to explain gelling capacity. Gelling capacity of all formulations were designated as + (gel formation takes after few minutes and disperse rapidly), ++ (immediate gel formation, remains un-dispersed for few hours) and +++ (immediate gel formation, the gel was remains for extend time). The results of all above parameters are shown in the Table 2.
- **Rheological studies:** The results of rheological study of prepared *in situ* gel confirms as the viscosity decreases with increase in angular velocity. The angular velocity and viscosity before and after gelation was tabulated in Tables 3 and 4, the corresponding rheograms are given in Figures 3 and 4. Results indicated that all formulations are having an optimum viscosity and all formulations were pourable at normal conditions.
- **Test for sterility*:*** All the formulations were found to be sterile when subjected to sterility study as per IP and no growth of any forms of microorganisms were observed in the formulations. There was no sign of appearance of turbidity and hence no evidence of microbial contamination.
- **In vitro drug release studies***:* The drug release data obtained for all the formulations and marketed eye drops are shown in Tables 5 and 6, respectively. The cumulative percent drug release of all formulations were 72.08±2.18% for PL_1 after 6 hours and 73.31±1.97%, 75.69±2.07% after 7 hours for PL_2, PL_3 and 82.78±1.85% , 78.39±1.80% for PL_4 and PL_5 respectively after 8 hours. Zero order, first order, Higuchi and Korsmeyer Peppas graphs are given in Figures 5 to 8. The results of regression analysis clearly indicated that all the formulations follow diffusion mechanism with highest R value for Higuchi curve. Further, all the formulations followed first order kinetics, since R value of first order for all the prepared formulations was found to be near one. The results are given in Table 7. This confirms the release of drug from matrix depends upon the concentration of drug. From the result of drug content, gelation pH, drug content, and drug release studies for all formulation, PL_4 formulation was selected as best formulation which has shown highest drug release till eight hours. Hence PL_4 formulation was chosen for stability studies.
- **Stabilities studies*:*** Stability study was conducted for PL_4 formulation for three months. Results of stability study revealed that there was slight decrease in drug content and this may be because of slight degradation of drug at elevated temperature. The stability studies of PL_4 formulation is indicated in Table 8.

Table 2: Appearance, pH, drug content and gelation studies data for all formulations.

Sr. No.	Formulations	Appearance	pH	Drug content (%)	Gelling capacity at 25°C	Gelling capacity at 37 °C
1.	PL1	Clear solution	6.36±0.026	96.62±0.190	-	+
2.	PL2	Clear solution	6.36±0.030	97.05±0.196	-	++
3.	PL3	Clear solution	6.39±0.025	96.38±0.850	-	++
4.	PL4	Clear solution	6.38±0.025	96.71±0.386	-	+++
5.	PL5	Clear solution	6.41±0.035	96.15±0.785	-	+++

The values presented are mean ±SD of 3 determinations.
- no gelation at 25°C
+ gelation within 50-60 seconds, dissolves rapidly
++ gelation within 60 seconds and remains stable for 3 hours
+++ gelation within 60 seconds and remains stable for 6 hours

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Table 3: Rheological profiles of all formulations (Before gelation)

Sr. No.	Angular velocity (rpm)	Viscosity (cps)				
		PL_1	PL_2	PL_3	PL_4	PL_5
1.	0.5	83.3±2.80	85.1±2.75	87.1±3.05	89.1±2.85	92.3±2.85
2.	1.5	69.3±2.85	71.0±2.80	73.1±2.85	75.5±3.05	78.4±2.75
3.	2.5	56.3±3.05	58.3±2.85	60.4±2.75	62.5±2.65	65.4±2.80
4.	5.0	35.0±2.75	37.3±2.70	39.1±2.80	41.3±2.90	44.1±2.70
5.	10.0	20.1±2.80	22.5±2.90	24.0±2.85	26.7±2.95	29.3±2.85
6.	20.0	12.2±2.90	14.2±3.10	16.4±2.90	**18.5±2.75**	21.0±2.80
7.	30.0	9.2±2.75	11.4±3.25	13.3±3.15	15.7±2.85	18.4±2.75
8.	40.0	7.3±2.80	9.4±2.75	11.4±3.20	13.0±2.80	16.1±2.80
9.	50.0	5.1±2.85	7.3±3.30	9.4±3.15	10.9±2.75	14.4±2.90

The values presented are mean ±SD of 3 determinations.

Table 4: Rheological profile of all formulations (After gelation)

Sr. No.	Angular velocity (rpm)	Viscosity (cps)				
		PL_1	PL_2	PL_3	PL_4	PL_5
1.	0.5	1476.6±5.77	1500.0±10.0	1516.6±5.77	1553.3±15.2	1586.6±5.77
2.	1.5	1370.0±10.0	1386.6±5.77	1413.3±15.2	1430.0±10.0	1473.3±15.2
3.	2.5	1283.3±15.2	1300.0±20.0	1320.0±10.0	1336.6±5.77	1380.0±10.0
4.	5.0	1180.0±20.0	1203.3±15.2	1216.6±5.77	1240.0±20.0	1286.6±11.5
5.	10.0	856.6±5.77	880.0±10.0	900.0±20.0	916.6±5.77	946.6±5.77
6.	20.0	630.0±10.0	646.6±5.77	670.0±10.0	**690.0±10.0**	730.0±10.0
7.	30.0	553.3±15.2	573.3±15.2	586.6±5.77	613.3±15.2	643.3±5.77
8.	40.0	456.6±5.77	480.0±10.0	503.3±15.2	516.6±5.77	546.6±15.2
9.	50.0	370.0±10.0	386.6±5.77	410.0±10.0	430.0±10.0	470.0±10.0

The values presented are mean ±SD of 3 determinations.

Table 5: *In vitro* drug release profile of Polyox 301 formulations

Sr. No.	Time (hours)	Cumulative percentage release in simulated tear fluid (STF) (%)				
		PL_1	PL_2	PL_3	PL_4	PL_5
1.	0	0	0	0	0	0
2.	1	22.24±1.05	24.96±0.92	26.78±1.05	33.83±1.08	29.66±1.02
3.	2	35.27±1.33	36.31±1.31	38.64±1.34	43.37±1.26	40.54±1.27
4.	3	47.44±1.60	47.18±1.52	49.44±1.54	52.67±1.39	49.37±1.41
5.	4	57.73±1.82	56.32±1.69	58.38±1.71	60.15±1.51	57.66±1.54
6.	5	65.90±2.00	63.64±1.83	65.84±1.85	67.31±1.61	64.57±1.66
7.	6	72.08±2.18	69.32±1.94	71.91±1.96	73.33±1.70	70.15±1.71
8.	7	-	73.31±1.97	75.69±2.07	78.59±1.78	75.17±1.83
9.	8	-	-	-	**82.78±1.85**	78.39±1.80

The values presented are mean ±SD of 3 determinations

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Table 6: *In vitro* drug release profile of marketed eye drops

Sr. No.	Time (hrs)	Cumulative percentage release in simulated tear fluid (STF) (%)
		Marketed eye drops
1.	0	0
2.	1	60.11±1.86
3.	2	78.54±1.79
4.	3	93.49±0.74

The values presented are mean ±SD of 3 determinations

Table 7: Mathematical kinetic models of formulations

Sr. No.	Formulations	Mathematical models (kinetics)				
		Zero order (R)	First order (R)	Higuchi model (R)	Korsmeyer Peppas model	
					(n)	
1.	PL_1	0.961	0.991	0.992	0.667	0.991
2.	PL_2	0.931	0.994	0.997	0.569	0.996
3.	PL_3	0.924	0.994	0.998	0.547	0.997
4.	PL_4	0.890	0.990	0.997	0.537	0.996
5.	PL_5	0.900	0.990	0.998	0.519	0.990

Table 8: Stability study for PL_4 formulation

Sr. No.	Time (Days)	Appearance	Drug content (%)	% CDR after 8th hours
1.	0	Clear solution	96.63 %	82.61 %
2.	30	Clear solution	95.79 %	81.47 %
3.	60	Clear solution	94.98 %	80.18 %
4.	90	Clear solution	94.19 %	78.96 %

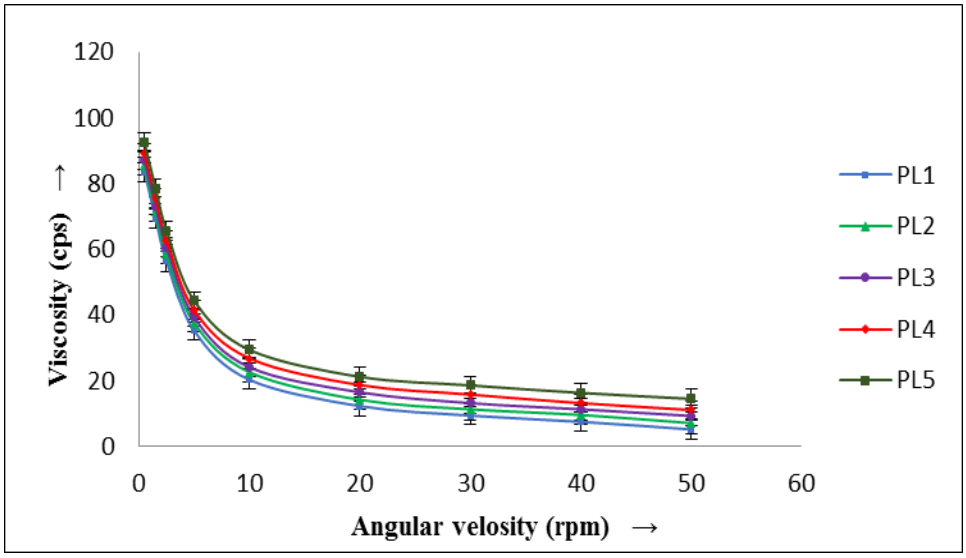

Figure 3: Rheograph of all formulations (Before gelation)

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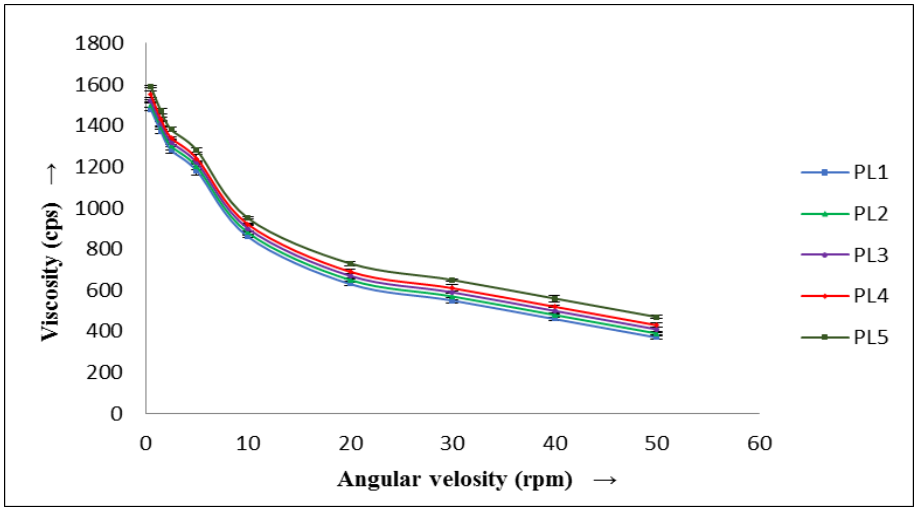

Figure 4: Rheograph of all formulations (After gelation)

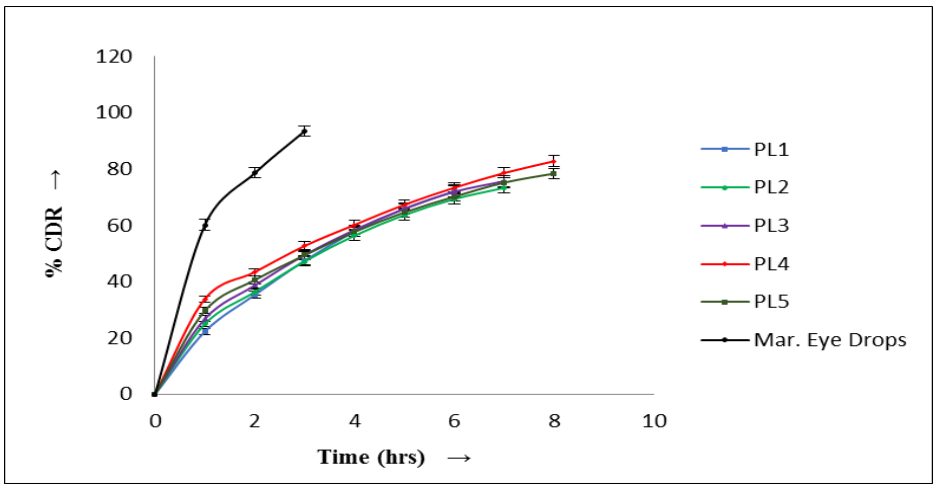

Figure 5: Comparative *in vitro* drug release profile of all formulations with marketed eye drops (Zero order kinetics)

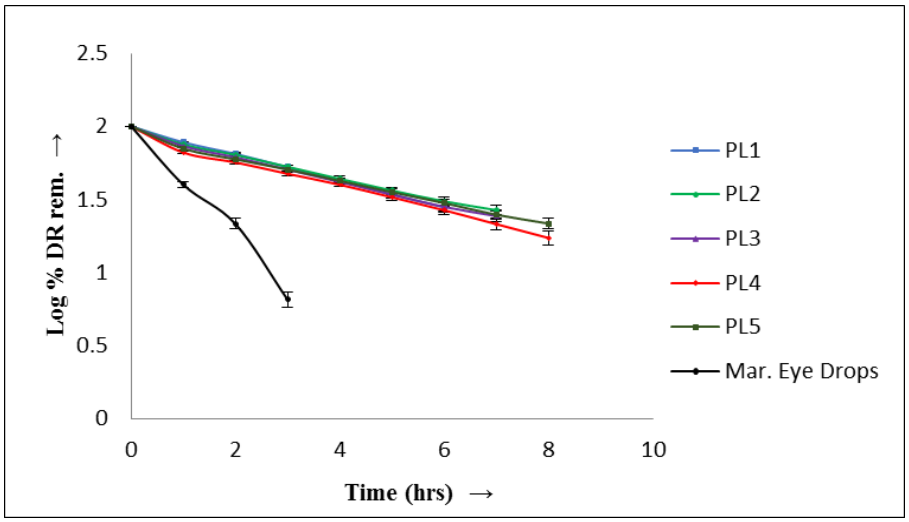

Figure 6: First order kinetics for all formulations and marketed eye drops

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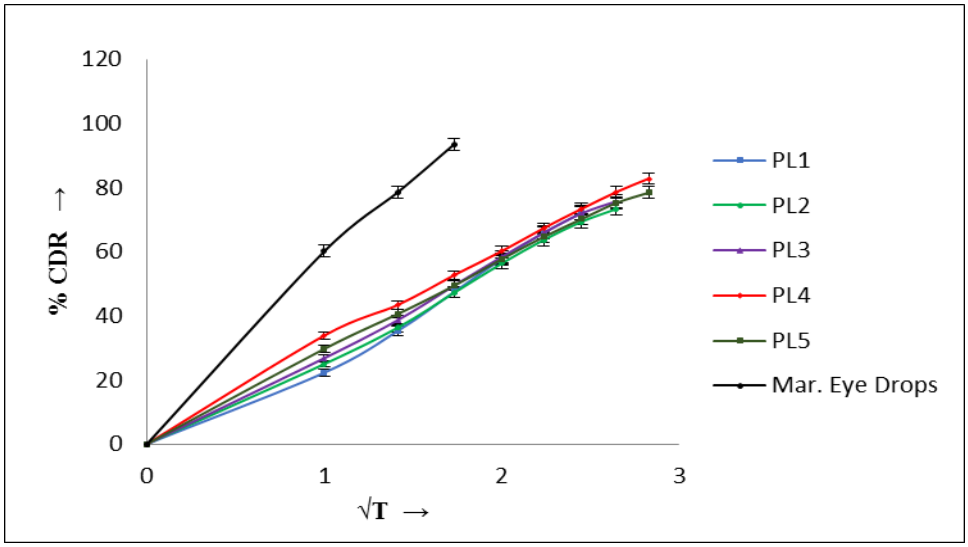

Figure 7: Higuchi release kinetics plots for all formulations

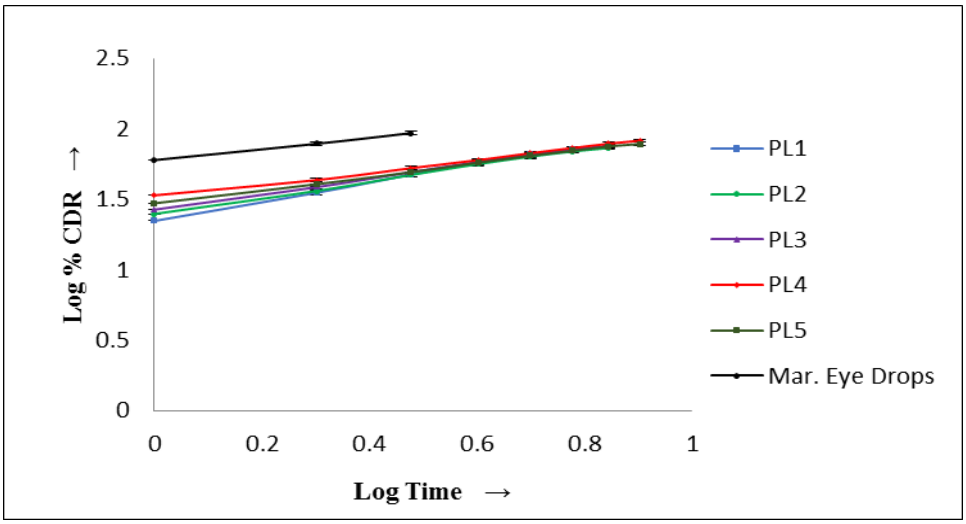

Figure 8: Korsmeyer Peppas (log-log) plots for all formulations

CONCLUSIONS

In the present work, an attempt was made to develop *in situ* gelling system of moxifloxacin with pH sensitive polymer. IR studies revealed that the drug and excipients were compatible with each other. Preparations were found to be clear, pH and drug content of all the preparations were found within the acceptable ranges. All formulations showed optimum viscosity and remained in gel form for few hours. They were pourable at normal conditions and viscosity increased after contact with STF. These formulations showed pseudoplastic flow behavior. The results of sterility test confirmed that all the formulations were sterile. Formulation PL_4 was found to show prolonged drug release for a period of 8 hours. The formulations were found to be stable in stability studies. Further detailed investigations are needed to establish *in vitro–in vivo* correlation to prove the bioavailability of prepared formulations.

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