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Formulation and characterization of thermosensitive in situ gel of moxifloxacin for ocular delivery

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Original article

Formulation and characterization of thermosensitive in situ gel of moxifloxacin for ocular delivery

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Abstract

The objective of this work was to develop temperature triggered *in situ* ophthalmic gel of moxifloxacin using Pluronic F-127 as a gelling agent to improve duration of contact with cornea. These systems are used as eye drops as they undergo reversible phase transformation within the cul-de-sac and thereby increase the ocular bioavailability and reduce the administration frequency. In the present study, moxifloxacin (a fluoroquinolone antibiotic) was used as a model drug. The work was planned to formulate ophthalmic *in situ* gel using Pluronic F-127 a gelling agent with hydroxyl propyl methyl cellulose K4M as viscosity imparting agent, sodium chloride as a tonicity modifier and benzalkonium chloride as a preservative. All the formulations were evaluated for appearance, pH, drug content uniformity, *in vitro* gelation studies, rheological studies, test for sterility, *in vitro* release studies and stability studies. Formulation PF2 was chosen as best formulation on the basis of its capacity to prolong the drug release till 8 hours. Percent drug content was 96.94±0.583%. The viscosity before gelation was 15.0±2.80 cps and after gelation was 683.3±15.2 cps at 20 rpm. The drug release was 78.65±1.71% after 8 hours for PF2 formulation.

Keywords: *In situ* ocular gel**,** Moxifloxacin, Pluronic F-127, Sol-to-gel phase transition

Introduction

Conventional ophthalmic delivery systems such as eye drops often result in poor bioavailability and therapeutic response because of production of more tear fluid. This leads to rapid elimination of the drug from the ophthalmic cavity. To maintain the drug concentration in the affected site, it is necessary to instill eye drops very often, which leads to poor patient compliance. Addition of more quantity of drug in the formulation is an attempt to increase the bioavailability of the drug, but it is dangerous if the drug solution drains off. Local ophthalmic drug delivery systems can be prepared using different 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. Various new type of ophthalmic drug delivery systems such as Ocuserts, ointments, suspensions, in situ gels etc.[1-5] have been developed to increase the ocular contact time and enhance the ophthalmic bioavailability.

Among the different novel ophthalmic drug delivery systems, *in situ* gelling systems have been found to be favorable dosage forms 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 aid 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

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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 and gellan gum). [2]

In the present study the method selected to prepare *in situ* gel was temperature sensitive gelation using commonly used polymer i.e. pluronic F-127, an environment-sensitive polymer. These preparations are able to increase or decrease its volume due to change in the temperature of the environment.[3]

Pluronic F-127 (PF-127), a thermosensitive polymer, consists of a mixture of polyoxy ethylene units (70%) and polyoxy propylene units (30%). These are liquid at low temperature i.e. below its low critical solution temperature (LCST) and get converted into gel when temperature increases i.e. above LCST. The aqueous solution of Pluronic F-127 (15% or more) is low viscous solution at temperature less than 4° C and gelation occurs when temperature increases i.e. at around 23°C. This phase conversion is reversible. That is to say at lower temperature, the preparation is in liquid state, which makes delivery to the ophthalmic cavity easy and accurate quantity can be delivered. Above this transition temperature, the solution becomes gel and is difficult to administer. The rise in the temperature alters the hydration capacity around the hydrophobic units, which in turn induces more interactions between the units.[6-7]

The purpose of the intended work was to develop temperature 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 topoisomerases and DNA gyrase, which in turn inhibits DNA synthesis.[3] 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 penetrates intracellularly. Approximately 52% of oral or intravenous dose is metabolized via glucuronide and sulphate conjugation.

Materials and Methods

Materials

Moxifloxacin was obtained as a gift sample from Centaur Pharma Pvt. Ltd., Goa, India. Pluronic F 127 was obtained from BASF Ltd., Mumbai, India. Other chemicals and reagents used in the study were of AR grade. Equipment used in the study are UV-Visible spectrometer (Shimadzu Corporation, Japan), IR-spectrometer (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 Equipment, Mumbai).

Methods

1. **Preformulation studies**

   Preformulation testing is the first step in development of dosage forms. Following tests were performed to identify and assess 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.[8-10]

2. **Method of preparation**

Pluronic F-127 *in situ* gel was prepared by the cold method. Required quantity of Pluronic F-127 and HPMC K4M (0.5 % w/v) was slowly added to the cold acetate buffer (pH 6.5) which contains 0.5% moxifloxacin and the contents were stirred constantly using temperature controlled magnetic stirrer. The prepared solution was stored in a refrigerator for about a day with occasional stirring until clear solution was formed. To the above solution 1% sodium chloride and 0.02% benzalkonium chloride were added. Acetate buffer (pH 6.5) was then added to make the volume up to 100 ml.[12-14] Different formulations were prepared as per Table 1.

Table 1: Composition of formulations

Sl. No.	Formulation code	Moxifloxacin (% w/v)	Pluronic F 127 (% w/v)	HPMC K_4M (% w/v)	Sodium chloride (% w/v)	Benzalkonium chloride (% w/v)
1.	PF_1	0.5	16	0.5	1.0	0.02
2.	PF_2	0.5	18	0.5	1.0	0.02
3.	PF_3	0.5	20	0.5	1.0	0.02
4.	PF_4	0.5	22	0.5	1.0	0.02
5.	PF_5	0.5	25	0.5	1.0	0.02

Acetate buffer (pH 6.5) - Quantity sufficient to 100 ml

3.Evaluation prepared in situ gel

- **Appearance:** Prepared formulations were tested for clarity by observing under highly illuminated white and dark backgrounds.[11]
- **pH:** The pH of the developed formulations were determined using digital pH meter. [12]
- **Drug content:** One ml of the preparation was diluted to 100 ml with pH 7.4 phosphate buffer solution. From the above solution 1 ml was withdrawn and diluted to 10 ml with pH 7.4 phosphate buffer. Concentration of moxifloxacin in all formulations was determined at 296nm using UV spectrophotometer.[12-14]
- ***In vitro* gelation studies:** This was determined by taking one drop of the preparation in a test tube which contains 2 ml of freshly prepared simulated tear fluid (STF). STF contained 0.670 gm of sodium chloride, 0.200 gm of sodium bicarbonate, 0.008 gm of calcium chloride 2H2O and purified water up to 100 ml. Temperature was maintained at 37 °C and time taken to form the gel and the gel to get dissolved was noted.[14-15] The results of pH, drug content and gelation studies are given in Table 2.
- **Rheological studies:** The study was performed using Brookfield viscometer and angular velocity was increased slowly from 0.5 to 50 rpm using spindle No. 62. The hierarchy of angular velocity was reversed and the mean dial reading was used to calculate the viscosity. Then the preparation was allowed to form gel in presence of STF and then again the viscosity determination was done. The temperature was maintained at 37±0.1°C.[11,16] The results of rheological studies before and after gelation are given in Tables 3 and 4. The rheograms of all the formulations are represented in figures 1 &2

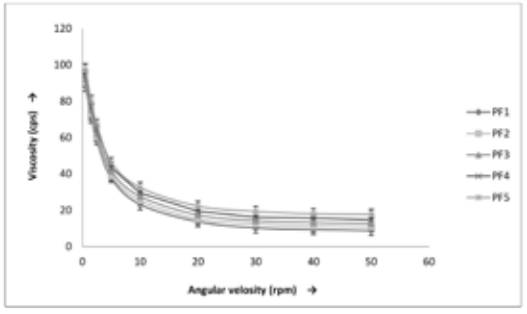

Figure 1. Rheograph of all formulations (Before gelation)

- **Test for sterility:** The sterility test was performed as per Indian Pharmacopoeia (IP). The test liquid was

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

Sr. No.	Formulations	Appearance	pH	Drug content (%)	Gelation studies	Gelation temperature. (°C)
1.	PF_1	Clear solution	6.78±0.025	96.49±0.515	++	36.23±0.450
2.	PF_2	Clear solution	6.83±0.035	96.94±0.583	+++	33.66±0.450
3.	PF_3	Clear solution	6.70±0.025	96.94±0.675	+++	32.76±0.351
4.	PF_4	Clear solution	6.61±0.030	96.49±0.515	+++	28.63±0.351
5.	PF_5	Clear solution	6.87±0.026	96.72±0.513	+++	26.23±0.450

The values presented are mean ±SD of 3 determinations.

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

Sr. No.	Angular velocity (rpm)	Viscosity (cps)				
		PF_1	PF_2	PF_3	PF_4	PF_5
1.	0.5	88.1±2.80	90.3±2.70	92.5±3.25	94.3±2.80	97.6±3.05
2.	1.5	71.3±3.15	73.3±3.35	75.1±2.75	77.0±2.75	80.3±2.70
3.	2.5	57.7±2.29	60.1±2.80	62.2±3.10	64.1±2.70	67.1±2.80
4.	5.0	37.0±2.20	39.0±2.90	41.4±2.95	43.3±2.85	46.1±2.65
5.	10.0	23.1±2.85	25.3±2.85	27.6±3.05	29.4±2.90	32.4±2.85
6.	20.0	13.5±2.70	15.0±2.80	17.3±2.85	19.3±2.80	22.1±2.75
7.	30.0	10.1±2.75	12.4±2.95	14.1±2.80	16.1±2.85	19.3±2.90
8.	40.0	9.1±2.61	11.4±3.20	13.3±3.15	15.5±2.70	17.9±2.80
9.	50.0	8.5±2.65	10.7±2.75	12.7±3.10	14.6±2.75	17.6±2.70

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)				
		PF_1	PF_2	PF_3	PF_4	PF_5
1.	0.5	1536.6±11.5	1573.3±15.2	1603.3±5.77	1646.6±5.77	1700.0±10.0
2.	1.5	1356.6±5.77	1396.6±5.77	1443.3±15.2	1480.0±10.0	1586.6±5.77
3.	2.5	1266.6±15.2	1310.0±10.0	1333.3±20.8	1376.6±15.2	1426.6±15.2
4.	5.0	1160.0±10.0	1223.3±15.2	1250.0±10.0	1286.6±5.77	1340.0±10.0
5.	10.0	853.3±5.77	900.0±20.0	933.3±5.77	980.0±10.0	1026.6±5.77
6.	20.0	620.0±20.0	**683.3±15.2**	706.6±15.2	756.6±5.77	813.3±15.2
7.	30.0	546.6±15.2	600.0±10.0	640.0±10.0	683.3±15.2	730.0±20.0
8.	40.0	473.3±5.77	503.3±5.77	546.6±5.77	590.0±10.0	636.6±5.77
9.	50.0	390.0±10.0	433.3±15.2	473.3±15.2	506.6±5.77	560.0±10.0

The values presented are mean ±SD of 3 determinations.

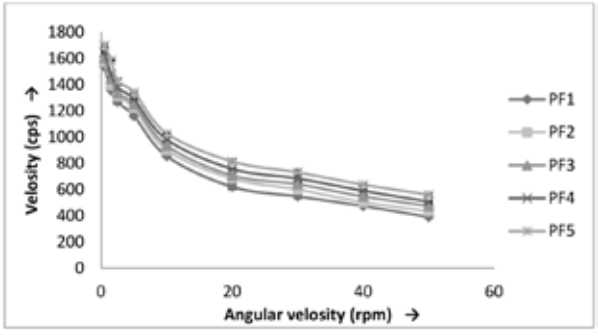

Figure 2. Rheograph of all formulations (After gelation)

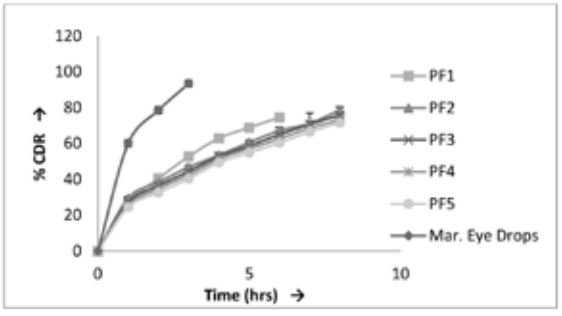

Figure 3. Comparative in vitro drug release profile of all formulations with marketed eye drops (Zero order kinetics)

aseptically transferred to fluid thioglycolate medium (20 ml) and soybean casein digest medium (20 ml) separately. The inoculated media were incubated for not less than 14 days at 30°C to 35°C for fluid thioglycolate medium and 14 days at 20°C to 25°C for soybean casein digest media.[12-16]

- **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.

Table 5: In vitro drug release profile of pluronic F 127 formulations

Sr. No.	Time (hours)	Cumulative percentage release in simulated tear fluid (STF) (%)				
		PF_1	PF_2	PF_3	PF_4	PF_5
1.	0	0	0	0	0	0
2.	1	27.20±0.97	29.46±0.99	27.17±1.06	25.49±0.68	24.48±0.91
3.	2	40.52±1.27	38.33±1.14	36.46±1.25	34.78±0.82	32.69±1.07
4.	3	52.76±1.46	46.75±1.25	44.39±1.39	42.46±0.88	40.44±1.23
5.	4	62.98±1.68	53.44±1.34	52.86±1.54	51.31±0.96	49.43±1.33
6.	5	68.89±1.78	60.52±1.43	58.61±1.64	57.03±1.00	54.91±1.42
7.	6	74.59±1.80	67.29±1.52	64.91±1.75	62.62±1.09	60.36±1.54
8.	7	-	73.74±1.61	70.66±1.85	68.63±1.10	66.57±1.60
9.	8	-	**78.65±1.71**	75.79±1.94	73.25±1.08	71.60±1.73

The values presented are mean ±SD of 3 determinations

Sr. No.	Time (hours)	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

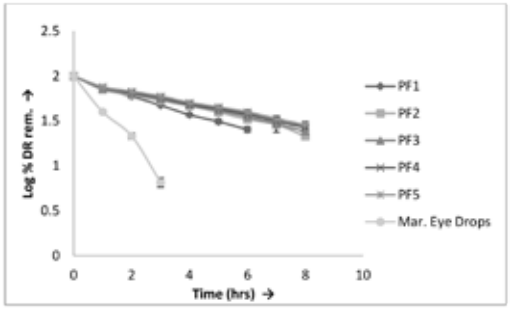

Figure 4. First order kinetics for all formulations and marketed eye drops

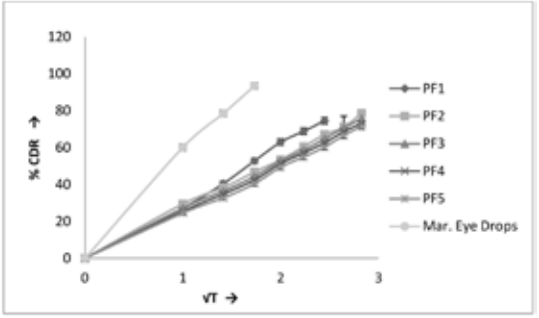

Figure 5. Higuchi release kinetics plots for all formulations

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 was withdrawn, suitably diluted and amount of moxifloxacin present at each time interval was calculated by using UV spectrophotometer at 296 nm.[10-12, 14-17] The cumulative drug release is given in Tables 5 and 6. Release kinetics data is given in Table 5. The various drug release models are represented in the figures 3, 4, 5 and 8.

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.[18-19]

Results and Discussion

The characterization studies on the properties of *in situ* gels have been performed to investigate whether the *in situ* gel would be advantageous to the conventional ophthalmic drops. The *in situ* gel was prepared by using varying concentration of temperature sensitive polymer i.e. PF-127. All the preparations were characterized for various evaluation tests.

Melting point of moxifloxacin was found to be 239°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 received drug sample was pure, as the observed frequencies

are within the range of reported one. Compatibility study was carried out IR frequencies are compared with other agents used in the formulations. The results of compatibility studies indicated that there was no interaction between contents of the formulation. IR spectra are shown in figures 6 and 7.

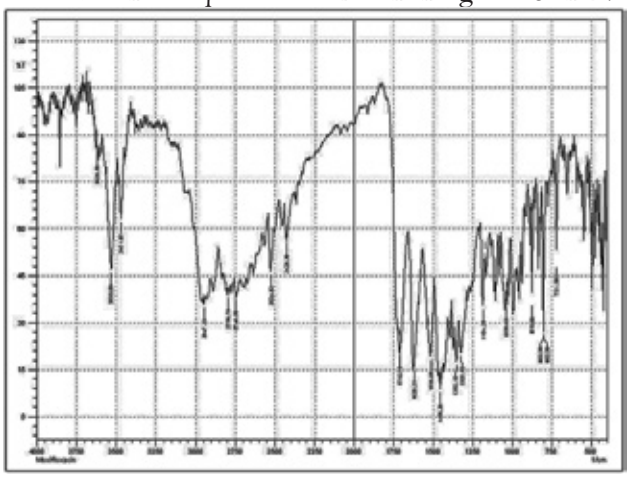

Figure 6. FTIR spectra of moxifloxacin

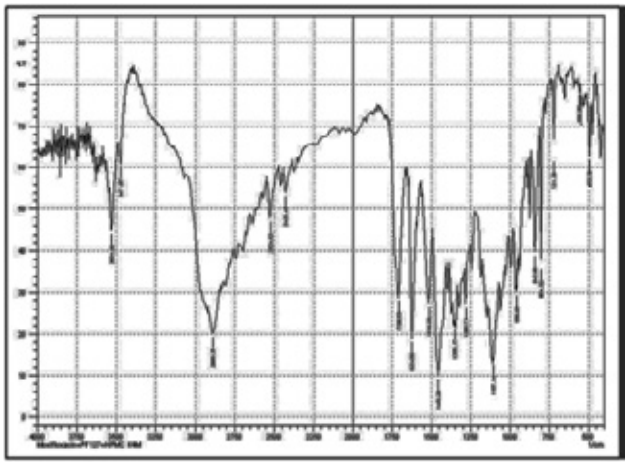

Figure 7. FTIR spectra of moxifloxacin, PF-127 and HPMC K4M

The results of sterility test indicated that all formulations were found to be sterile as there was no sign of appearance of turbidity and hence no evidence of microbial contamination. The drug content uniformity data has shown that all the formulations were found to be uniform in content. 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 undispersed for few hours) and +++ (immediate gel formation, the gel was remains for extend time). Gelation temperature of formulations was found to be in the range between 26.23±0.450°C to 36.23±0.450°C. The results of gelling capacity and gelation temperature indicates that all the formulations convert sol to gel when it is exposed to a favorable environment The results of all these parameters was recorded in Table 2.

The results of rheological study of prepared *in situ* gel confirms that 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 1 and 2. Results indicated that all formulations are having an optimum viscosity and all formulations were pourable at normal conditions.

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 74.59±1.80% for PF1 after 6 hours and 78.65±1.71%, 75.79±1.94%, 73.25±1.08% and 71.60±1.73% for PF2, PF3, PF4 and PF5 respectively after eight hours. Zero order, first order, Higuchi and Korsmeyer Peppas graphs are given in figures 3 to 8. The results of regression analysis clearly indicated that all the formulations follow diffusion mechanism with

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)	(R)
1.	PF_1	0.930	0.994	0.997	0.574	0.996
2.	PF_2	0.933	0.990	0.992	0.525	0.991
3.	PF_3	0.924	0.990	0.998	0.542	0.996
4.	PF_4	0.928	0.991	0.998	0.516	0.997
5.	PF_5	0.936	0.991	0.997	0.526	0.994

highest R value for Higuchi curve. Further, all formulations followed first order kinetics, since R value of first order for all the prepared formulations was found to be near one. This confirms the release of medicament from matrix depends upon the concentration of drug. From the result of all parameters i.e. drug content, gelation temperature, and drug release studies for all formulation, PF2 was coined as best formulation which has shown highest drug release up to eight hours. Hence, PF2 formulation was chosen for stability studies.

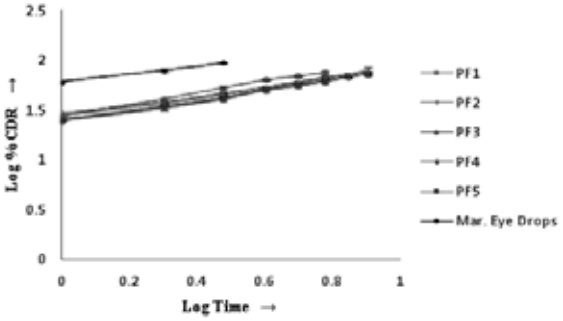

Figure 8. Korsmeyer Peppas (log-log) plots for all formulations

Table 8. Korsmeyer Peppas (log-log) plots for all formulations

Sr. No.	Time (Days)	Appearance	Drug content (%)	% CDR after 8th hours
1.	0	Clear solution	96.85	78.57
2.	30	Clear solution	96.05	77.31
3.	60	Clear solution	95.15	76.13
4.	90	Clear solution	94.55	74.87

Stability study was conducted for PF2 formulation for three months. The results of stability study are recorded in Table 8. 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.

Conclusions

In the present work, an attempt was made to develop *in situ* gel of moxifloxacin with temperature sensitive polymer. IR spectroscopy studies revealed that drug and excipients were compatible with each other. Preparations were found to be clear and pH and drug content of all the preparations were found within the acceptable ranges. Formulation PF1 remained in gel form for a few hours. All formulations showed optimum viscosity. 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 PF2 was found to show prolonged drug release for a period of eight 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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