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Research Article

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OPTIMIZATION OF MOXIFLOXACIN IN-SITU OCULAR GEL BY 3^2 FACTORIAL DESIGN

Ashir TP*, Ganesh N.S and Vineeth Chandy

Abstract

Moxifloxacin HCl is a fourth generation fluoroquinolone antibiotic groups. The present study was aimed to formulate and evaluate Moxifloxacin *in-situ* gel by 3^2 factorial designs for effective ocular delivery and to improve patient compliance. This formulation is based on the concept of temperature triggered *in-situ* gelation using different ratio of Pluronic F127 as gelling agent and Hydroxy Propyl Methyl Cellulose as viscosity enhancer. A 3^2 factorial design was used to design and develop ocular *in-situ* gel formulation. Here two factors were evaluated in each three levels. In the present study concentration of Pluronic F127 and HPMC were selected as independent variables. The percentage drug release at 8th hour, gelling capacity and viscosity were chosen as dependent variables. The prepared *in-situ* ocular gels were tested for their physicochemical characteristics such as, FT-IR studies, physical appearance, Drug content determination, Clarity, pH, Gelling capacity, Gelling temperature, Viscosity, Isotonicity testing, antimicrobial study, Stability study and *in-vitro* diffusion studies. Formulation F2 (Pluronic F127 (30mg) HPMC (15mg)) showed a better drug release of 96.65% at the end of 8th hour.

Key Words: Moxifloxacin HCl, Pluronic F127, HPMC, 3^2 factorial designs, FTIR, In-vitro Drug Release

INTRODUCTION

Eye drops that are conventional ophthalmic delivery systems often result in poor bioavailability and therapeutic response, because high tear fluid turnover and dynamics cause rapid precorneal elimination of the drug. In the ophthalmic drug delivery systems, protective barriers of eye lead to low absorption of drug and it leads to poor bioavailability of therapeutic drugs.

In the present study an attempt will be made to formulate and evaluate Moxifloxacin HCl *in-situ* gel by 3^2 factorial designs for effective ocular delivery and to improve patient compliance. A 3^2 factorial design was used to design and develop ocular *in-situ* gel formulation. Here 2 factors are evaluated in each 3 levels. In the present study concentration of Pluronic F127 and HPMC were selected as independent variables. The percentage drug release at 8th hour, gelling capacity and viscosity were chosen as dependent variables
The objectives of the study were:

- To maintain the therapeutic drug concentration in eye for prolonged period of time.
- To reduce the frequency of drug administration. Improve patient compliance.
- To formulate and evaluate Moxifloxacin *in situ* ocular gel by 3^2 factorial design

MATERIALS AND METHODS

Sl. No	Materials	Source
1	Moxifloxacin HCl	Swapnaroop drugs & Pharmaceuticals, Maharashtra
2	Pluronic F127	Yarrow chemicals Pvt Ltd, Mumbai
3	Hydroxypropyl methylcellulose	Loba Chemie Pvt. Ltd, Mumbai
4	Sodium chloride	Yarrow chemicals Pvt Ltd, Mumbai
5	Benzalkonium Chloride	HiMedia laboratories Pvt. Ltd, Mumbai

Pre-formulation Parameters

Melting point determination of Moxifloxacin HCl

Melting point of Moxifloxacin HCl was determined by using Thale's tube method by taking a small amount of drug in a capillary tube closed at one end and placed in Thale's tube containing liquid petroleum and temperature at which drug melts was recorded. This was performed in triplicates and average value was reported.

Solubility of Moxifloxacin HCl

Solubility of Moxifloxacin HCl was performed in various solvents like ethanol and water. Accurately weighed one gm of drug was transferred in a clean and dry test tube followed by addition of the solvents individually and shaken vigorously and the solubility of drug was checked visually.

Infrared spectral studies

Method: In this technique, approximately 1 mg of the Moxifloxacin HCl was allowed to mix with about 100 mg of KBr (which is transparent to IR) in the ratio of 1:100 and then thoroughly mixed in a mortar. The mixture was pressed in a pellet die manually and placed it in a Fourier transform infrared (FTIR) spectrophotometer (Shimadzu corporation 8400S, Japan).

Experimental Design

Ocular *in-situ* gels were formulated using varying concentration of gelling agent and viscosity enhancers. Formulations were prepared as mentioned bellow

Effect of different concentration of gelling agent and viscosity enhancers on in-situ gel

A technique of 3^2 factorial design taking 2 variables namely gelling agent (Pluronic F127) and Viscosity enhancer (HPMC) at 3 different levels affecting the % drug release, Viscosity and Gelling capacity determination were used to design the experimental batches for the preparation of ocular gel.

Department of Pharmaceutics, T. John College of Pharmacy, Gottigere, Bangalore, Karnataka – 560083
Correspondence and Reprint Requests: Ashir TP

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A factorial design evaluating 2 factors at all combinations for each factor would result in a full factorial design consisting of $3^2 = 9$ runs. The addition of center points allows for detection of nonlinearity in the responses. The total number of runs becomes 9 + 5 runs =14 runs.

The center points were run 6 times to get an estimate of experimental or pure error. F test was used to compare the variance among the treatment means with the variance of individuals within the specific treatments.

Statistical Design

A commercially available software program was used (Design Expert, Version 10, Stat-Ease Inc, Minneapolis, MN). The experimental design chosen was response surface, 2-factor, 3-level; 14 formulations were prepared. Run order was randomized to protect against the effects of time-related variables and also to satisfy the statistical requirement of independence of observations. Analysis of variance (ANOVA) and all statistical analyses were also performed using the Design Expert software. The F value was then calculated by comparing the treatment variance with the error variance. The multiple correlation co-efficient was calculated, which is a measure of the amount of variation about the mean, which is explained by the model. All assumptions underlying the ANOVA were checked.

Table 4.3 Factors and levels for the 3^2 Factorial design of Ocular *in-situ* gel

Independent variable	Levels		
	Low(mg)	Medium(mg)	High(mg)
Pluronic- F127	10	20	30
HPMC	5	10	15

Dependent variable
Y1 % Drug Release
Y2 Gelling Capacity
Y3 Viscosity

Method of preparation of in-situ ocular gel of Moxifloxacin HCl

Methods

Cold Method

HPMC was dissolved in water was added to Pluronic solution (prepared by dispersing Pluronics over the distilled water with continuous stirring(500rpm) and it is kept in refrigerator (4 degree) until the Pluronics dissolves (Magnetic stirrer) (24Hour)

Mixed both solution @300 rpm on magnetic stirrer, 500mg drug solution was prepared by dissolving in water was prepared and added to above solution.

0.02% Benzalkonium chloride solution was added to above solution as preservative and the pH was adjusted to 7.4 using 0.5M NaOH. Which is then sterilized in Autoclave @121 degree for 20 minute.

Formulation of Moxifloxacin HCl *in-situ* ocular gel

Evaluation of in-situ ocular gel

Drug content determination

The amount of drug contained in the Ocular gel was determined by dissolving 1ml of the formulation in 9 ml of water and the volume was made up to 100 ml with water. The mixture was analyzed by a UV-Visible spectrophotometer at 290 nm against water as a blank.

Clarity

Clarity examination involves the visual assessment of formulation in suitable lighting on white and black background. The examination is done for all the formulations of *in-situ* gels before and after gelling occurs.

pH

The pH of the formulations was found to be satisfactory and was in the range of 5.4-7.2, as per the limit. The formulations were liquid at room temperature and at the pH formulated. Terminal sterilization by autoclaving had no effect on the pH.

Gelling capacity

The gelling capacity of the prepared formulation was determined by placing a drop of the formulation in a vial containing 2 ml of freshly prepared simulated tear fluid and visually observed. The time taken for its gelling was noted. Gelling capacity of all formulations, which is depicted as Gelation was immediate and remains for 6 hours (++) and Gelation was immediate and remains for 8 hours (+++).

Gelling temperature

The gelation temperature was determined by placing the solution in test tube: the test tube was dipped in water bath whose temperature was maintained at 37 ± 5°C for 2 min. The temperature at which solution was converted to gel was noted down by placing the thermometer in the test tube.

Viscosity

The viscosity of the formulation after addition of simulated lachrymal fluid was evaluated by a Brookfield DV III Programmable Rheometer, using increased rotation speed. All the selected formulations were shear thinning, exhibiting pseudo plastic behavior. All the formulations were liquid at room temperature and underwent rapid gelation upon raising the pH to 7.4. The viscosity of each formulation at different rpm was recorded.

Isotonicity testing

Formulations (1 ml) was mixed with few drops (4 drops) of blood and observed under microscope at 45X magnification and the shape of the blood cell was compared with the standard marketed ophthalmic formulation.

Sl no.	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14
1	Moxifloxacin HCl	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2	Pluronic F127	30	30	30	30	30	10	10	10	20	20	20	10	10	10
3	HPMC	5	15	10	10	5	10	15	5	5	15	10	10	5	15
4	Sodium chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
5	Benzalkonium Chloride	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
6	Distilled Water	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Sterility testing was done by direct inoculation method using Fluid Thioglycolate and Soyabeen Casien Digest media and incubated for 7 days under daily observation.

Antimicrobial study

Antimicrobial activity was determined by agar diffusion test employing cup plate technique. The drug was allowed to diffuse through a solid agar medium. The standard minimum inhibitory concentration (MIC 2 μg/ml) of control and developed formulations containing Moxifloxacin were prepared. A total of 60 ml of nutrient agar media was prepared and sterilized at 15 lb/sq-inch pressure for 18 minutes in an autoclave; 0.5 ml of microorganism suspension was poured into the above medium which is maintained at temperature of 52°C to 58°C. This will be done in an aseptic condition. Immediately 20 ml of the microbial agar suspension was poured into each petriplate. After solidification of the media, sterile solutions of marketed product A (standard solutions) and the developed formulations diluted suitably with sterile distilled water (test solutions) were poured in to the cup of sterile nutrient agar Petri plates. This was previously seeded with test organisms (*Escherichia coli* and *Staphylococcus aureus*). After allowing diffusion of the solutions for 2 hours, the agar plates were incubated at 37°C for 24 hours. The Zone of inhibition (ZOI) was measured around each cup and compared with that of control. The entire operation was carried out in a laminar airflow unit. Each formulation solution was tested in triplicate. Both positive and negative controls were maintained throughout the study.

Stability study

Optimized sterile formulation was subjected to stability testing. Sterile optimized ophthalmic formulation was filled in glass vials, closed with gray butyl rubber closures and sealed with an aluminum caps. The vials contain optimized formulation were kept in stability chamber, maintained at 40 ± 2°C and 75 ± 5 % RH for one month. Samples were withdrawn and estimated for drug content, pH, visual appearance, gelling capacity and *in vitro* drug release.

In-vitro diffusion studies

In-vitro diffusion study of *in-situ* gel was carried out by using Franz diffusion cell. The formulation placed in donor compartment and freshly prepared simulated tear fluid in receptor compartment. Between donor and receptor compartment dialysis membrane is placed (0.22µm pore size). The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37°C ± 0.5°C. 1ml of sample is withdrawn at predetermined time interval of 1hr for 8 hrs and same volume of fresh medium is replaced. The withdrawn samples are diluted to 10ml in a volumetric flask with respective solvent and analyzed by UV spectrophotometer at respective nm using reagent blank. The drug content calculated using the equation generated from standard calibration curve. The % cumulative drug release (%CDR) calculated.

Storage-Physical Stability Evaluation of in-situ ocular gel

Stability of a drug has been defined as the ability of particular formulation in specific container to remain within its physical, chemical, therapeutic and toxicological specification.

Factor affecting stability

Extrinsic: Temperature, light, gases, moisture
Intrinsic: pH, complexation, microbial growth
Boundary: Container composition, porosity, dosage form interaction.

Stability testing is an integral part of formulation development. It generates information on which to base proposals for shelf lives of drug substance and products. And their recommended storage conditions. Stability data also are a part of the dossier submission to regulatory agencies for licensing approvals.

Salient features of ICH guidelines

- The stability test should be conducted using the containers and closures proposed for storage and distribution.
- The stability plan must include different types of containers and closures such as those used for marketing, physician and promotional samples and bulk storage.

However, for bulk containers testing in prototype container that simulates the actual packaging is allowed in ICH and FDA guidelines.

- A sampling frequency of every 3 months during the first year, every 6 months during the second year and then annually for drug substances and products stored for real time testing.
- At least two containers are required to be sampled during the stability study.
- To predict the shelf life of the dosage form for clinical zone III and IV, the predictive factor is 3.3 at 30°C (6 months at 40°C corresponds to 20 months at 30°C).

Procedure: *In-situ* was evaluated for drug retentive potential at 25±2⁰C for a period of 90 days. The *In-situ* gels were kept in containers. Sample were withdrawn periodically and analyzed for the physical appearance and drug content.

RESULT AND DISCUSSION

In-vitro Gelation Study:

Sl.No	Formulation	Gelling capacity
1	F1	++
2	F2	+++
3	F3	++
4	F4	+++
5	F5	++
6	F6	+++
7	F7	+++
8	F8	++
9	F9	++
10	F10	+++
11	F11	++
12	F12	+++
13	F13	++
14	F14	++

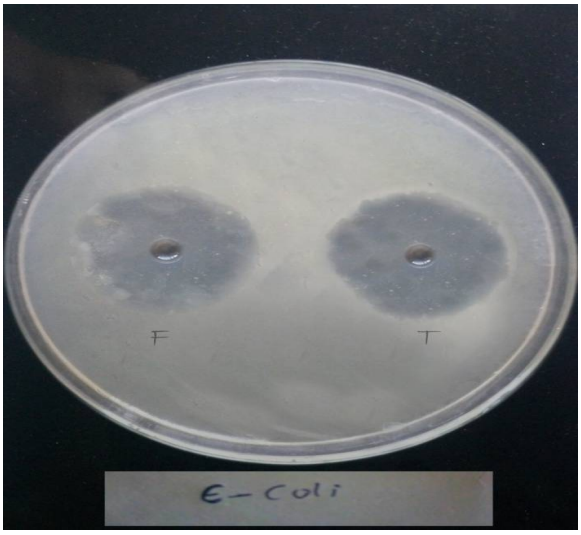
Viscosity of Formulations (F1 - F14)

Sl no	Formulation	RPM	Viscosity (p)
1	F1	50	372
2	F2	50	430
3	F3	50	395
4	F4	50	390
5	F5	50	394

6	F6	50	399
7	F7	50	399
8	F8	50	388
9	F9	50	424
10	F10	50	389
11	F11	50	420
12	F12	50	426
13	F13	50	393
14	F14	50	385

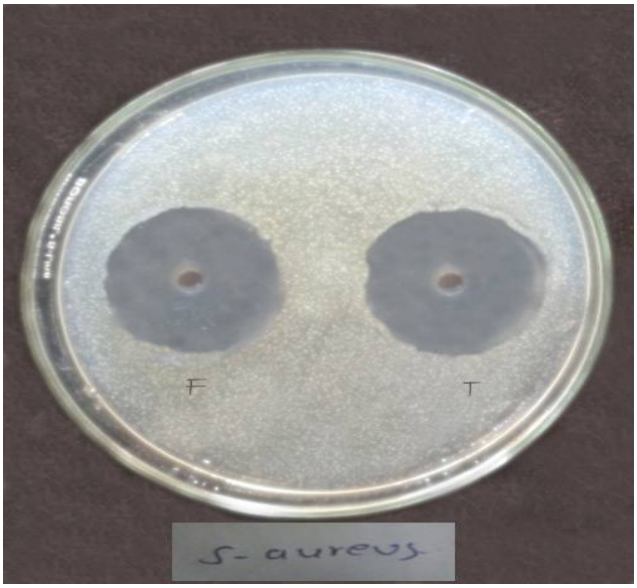
Antimicrobial Study

F- Formulation
T- Marketed Product



ZOI of formulation and marketed product Seeded with Escherichia coli

F- Formulation
T- Marketed Product



ZOI of formulation and marketed product Seeded with Staphylococcus aureus

Experimental design

	Factor 1	Factor 2	Response 1	Response 2	Response 3
Std Run	*Pluronic F127* mg	*HPMC* mg	*% Cumulative drug release Hrs*	*Gelling capacity*	*Viscosity cp*
1	1	30	5	93.21	7	371
1	2	30	15	96.65	7.5	430
1	3	30	10	95.56	7.2	380
2	4	30	10	94.45	7.7	382
2	5	30	5	90.48	7.4	362
3	6	10	10	72.84	7.8	350
3	7	10	15	75.32	8	362
3	8	10	5	70.15	7	340
4	9	20	5	85.62	7.1	365
4	10	20	15	87.36	7.8	372
4	11	20	10	86.74	7.1	368
5	12	10	10	74.12	7.6	350
5	13	10	5	72.21	7.3	343
5	14	10	15	78.32	7.5	361

Observed Response in 3² Factorial Design for in-situ ocular gel

The result depicts that variables chosen have strong influence on the selected responses, viscosity, gelling capacity and percentage cumulative drug release values were in the range of 340-430, 7-8 and 70.15-96.65% respectively.

The application of factorial design yielded the following regression equations.

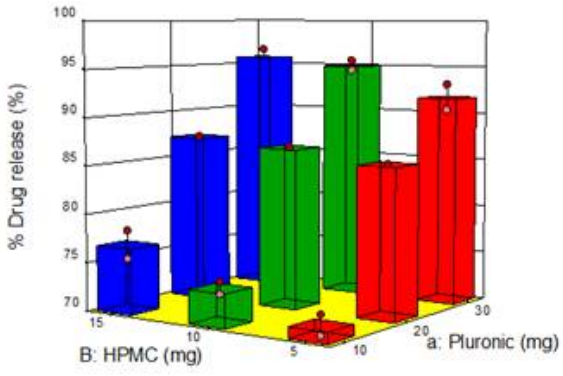
Viscosity= +75-0.29*HPMC[1]+0.026*HPMC[2]
Gellingcapacity=+366.39-15.39*Pluronic[1]+1.94*Pluronic[2]-8.72*HPMC[1]-0.056*HPMC[2]-0.78* Pluronic [1]HPMC[1]+5.39* Pluronic [1]HPMC[1]-0.94* Pluronic [1]HPMC[1]-0.28* Pluronic [2]HPMC[2]

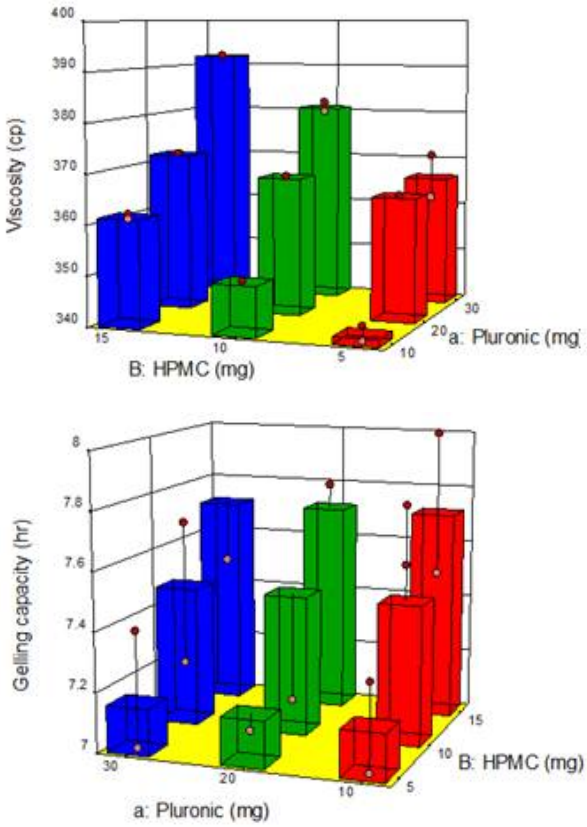
%Drugrelease=+84.8711.05*Pluronic[1]+1.70*Pluronic[2]1.99*HPMC[1]+0.20*HPMC[1]0.66*Pluronic[1]HPMC[1]+1.04*Pluronic[2]HPMC[1]-0.55*Pluronic[1]HPMC[2]-0.038*Pluronic [2]HPMC[2]

Where negative values indicate a negative effect of a specific variable on the response factors and positive values indicate a positive effect of a specific variable on the response factors. The polynomial regression results were expressed using 3-D graphs and contour plots.

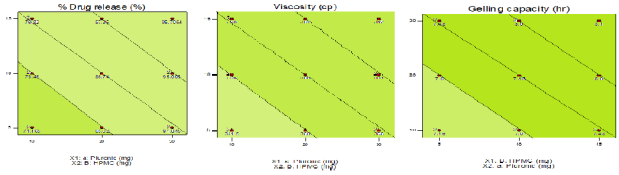
In-vitro diffusion studies

In-vitro diffusion of the formulation is showed in the table. The drug release data were subjected to various pharmacokinetic parameters like Zero order, First order, Higuchi square root and Kromeyer-Peppas model to know the pattern of the drug release table. The formulation F2 showed good sustained release for the period of 8 hours and finally comparative study with marketed formulation was done. This relationship between the formulation ingredients (independent variables) and Viscosity, Gelling capacity and drug diffusion rates (dependent variables) was elucidated using 3D graphs (Figure 5.15).



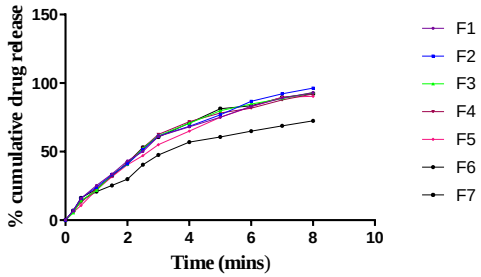


Three-dimensional response surface plot depicting the impact of Pluronic F127 and HPMC on % drug release respectively

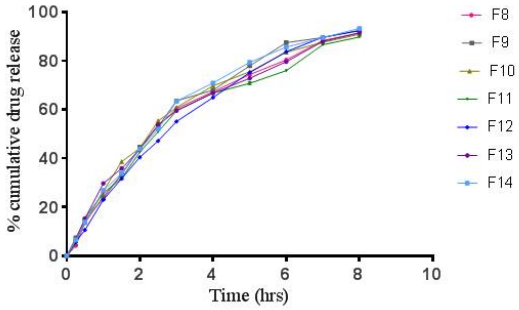


Counter Plot Showing impact of Pluronic F127 and HPMC on % drug release respectively

There is no significant contribution by eliminated terms from the equation on the prediction of Cumulative percentage drug release. Results of regression analysis showed coefficients of Pluronic and HPMC bearing a positive sign, i.e., with increase in the concentration of these factors show positive effect on percentage cumulative drug release. This is due to, with increase in the concentration of Pluronic and HPMC, the gelling capacity increase which leads to delay in diffusion of gel from the eye and thus increases the drug release.The Response surface plots and contour plots exhibits, as the concentration of HPMC and Pluronic F127 increases, the Viscosity and Gelling capacity increases respectively.



***in-vitro* diffusion profile of Formulations (F1-F7)**



***in-vitro* Diffusion profile of Formulations (F8-F14)**

CONCLUSION

Ocular therapy by *in-situ* gelling system had significantly improved the pre-corneal residence time of the drug. Several new preparations have been developed for ophthalmic use not only to prolong the contact time of the drug at ocular surface, also to slow down the elimination of the drugs from the eye.

In recent years, large number of experimental designs has been frequently applied to the optimization of formulation, considering their advantages such as reduction in the number of experiments that need be executed resulting in lower excipient consumption and considerably less laboratory work. After doing the entire work I concluded that,

- Moxifloxacin HCl is an antibiotic which was selected for the preparation of *in-situ* ocular drug delivery system as it complies with physicochemical properties required to permeate cornea.
- The preformulation studies involving description, solubility, melting point, partition coefficient of the drug were found to be comparable with the standard.
- The in-situ gels were prepared by Temperature triggered method.
- The in-situ gels were subjected for following evaluation parameters such as physical appearance, pH, Gelling temperature, Gelling capacity, Viscosity, Eye irritation test, Isotonicity testing, Antimicrobial study, diffusion studies and Stability study. All the parameters shows were within the limits.
- FT-IR study shows that there is no incompatibility between drug and polymers.
- The formulation F2 shows optimum diffusion in concentration independent manner. The above formulation gave maximum drug diffusion 96.65% at the end of 8th hours.

- Hence from the above result we can conclude that, it is possible to formulate more optimized *in-situ* ophthalmic gel Moxifloxacin HCl using Pluronics and HPMC for the treatment of various bacterial infections.

As an extension of this work pharmacokinetic studies, *in-vivo* studies on higher animal models and controlled clinical studies on human subjects may be carried out in future

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