Original Article

NEWER OPHTHALMIC IN SITU GEL OF MOXIFLOXACIN HYDROCHLORIDE: OPTIMIZATION USING BOX BEHNKEN STATISTICAL DESIGN

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ABSTRACT

Objective: The present research work aims at describing the formulation and evaluation of the ocular delivery system of moxifloxacin hydrochloride (MH) based on the concept of ion sensitive in situ gelations.

Methods: In situ gel was prepared by a hot method using 0.6% of gelrite, 0.25% hydroxypropylmethylcellulose (HPMC K_4M) and 0.023% tamarind gum as bioadhesive polymers for sustained drug release. Optimization was done by Box Behnken Design with different concentration of gelrite (X_1), HPMC K_4M (X_2) and tamarind gum (X_3) as independent variables. In situ gel was optimized based on mucoadhesion index (Y1), Gel strength (Y2) and *in vitro* drug release (Y3). Influence of the quantitative variable on the dependent variable was predicted by a polynomial equation.

Results: Infrared spectroscopy excluded any interaction between drug and excipients. The selected independent variables significantly influenced the responses and were able to sustain the drug release. The prepared gel with a pH of 6.8 to 7.4 exhibited non-newtonian flow with no ocular irritation. The formulation remained stable with no change in pH and viscosity after 30 d of stability study.

Conclusion: Thus, moxifloxacin hydrochloride (MH) in situ gel is a viable alternative to a conventional delivery system with the properties of sustained drug release, prolonged ocular retention, and improved corneal penetration.

Keywords: Moxifloxacin hydrochloride (MH), Box-Behnken Design, Gelrite, HPMC K_4M, Tamarind gum

INTRODUCTION

Development of suitable drug delivery systems for ocular therapy is one of the major problems being faced by pharmaceutical scientists. Eye, being the most interesting organ due owing to its drug disposition characteristics is considered a convenient route for the topical application of drugs [1]. But the attainment of an optimal concentration at the site of action circumventing the protective barriers of the eye without causing permanent tissue damage offers a significant challenge to the formulator.

Conventional ocular dosage forms result in poor bioavailability due to tear production, nonproductive absorption, transient residence time, and impermeability of corneal epithelium. These physiological and anatomical constraints deliver only a small fraction of the instilled dose of ocular therapeutics and less than 1% is effectively absorbed and reaches the internal anterior tissue of the eyes [2].

Several methods for prolonging the contact time between drug and corneal–conjunctival epithelium are investigated to increase the drug bioavailability. Various ophthalmic products, such as inserts, ointments, suspensions, and aqueous gels, have been developed to enhance ophthalmic bioavailability. These ocular drug delivery systems, however, have not been used extensively because of some drawbacks, such as blurred vision and poor patient compliance.

As the ocular efficacy of topically applied drugs is influenced by the corneal contact time, in situ gel is the most common method of improving the ocular availability of drugs. In situ gel system is formulated as liquid preparation suitable to be instilled into eyes which upon exposure to the physiologic environment changes to gel, thus prolongs the precorneal residence time and enhances the ocular bioavailability of the drug.

Topical administration of antibacterial medication to the conjunctival sac is usually an effective avenue for treating bacterial conjunctivitis, keratitis, and uveitis but requires frequent instillation. Moxifloxacin, an 8-methoxy fluoroquinolone (4th generation), having broad-spectrum antibiotic activity, with efficacy against various gram-positive and gram-negative microorganisms through inhibition of DNA gyrase and topoisomerases IV and is indicated for severe infections. To increase the bioavailability of moxifloxacin hydrochloride in situ gelling systems can be highly advantageous [3].

In situ gels consist of polymeric networks that can absorb large quantities of water while remaining insoluble in aqueous solutions due to chemical or physical cross-linking of individual polymer chains [4-5]. Increase in solution viscosity by using polymers improves retention of product on the corneal surface. More recently, the newer approach to improve mechanical strength and enhance precorneal resident time of in situ gel is based on the use of mucoadhesive polymers. The principle for the use of bioadhesive polymers relies on their ability to interact with the mucin present on the eye surface. The present investigation uses the ion-sensitive gelling mechanism with HPMC K_4M and tamarind gum as potential bioadhesive polymers for the formulation of hydrogels. The formulation also consists of gellan gum as an ion-sensitive polymer which is having good gel strength and biocompatibility [6-8].

In this regard, the present investigation is intended to characterize and evaluate the in situ gel of moxifloxacin hydrochloride, using bioadhesive polymers for sustaining drug release for a longer period. Optimization of the formulation was done using Box-Behnken statistical design (Design Expert® 8.0.7.1). The formulation variables that could affect the release rate and absorption of the drug in topical formulations, such as mucoadhesion strength, gelation strength, and the drug concentration in the formulations, were studied. In addition, the in vivo performance of gel formulation was assessed on the basis of the ocular irritancy in the rabbit's eye [9].

MATERIALS AND METHODS

Materials

Moxifloxacin hydrochloride (MH) was gifted by MARCK Bioscience Ltd, Kheda. Hydroxy-propylmethylcellulose (HPMC K_4M) was

supplied from colorconasia Pvt. Ltd. Gellan gum (Gelrite) and mucin were supplied from Hi-media Laboratories Pvt. Ltd. Tamarind gum was received from Shivam Exim Ltd, Ahmedabad.

Compatibility study

The physicochemical compatibility between moxifloxacin hydrochloride (MH) and polymers was studied using Fourier transforms infrared spectroscopy (FTIR) and Differential scanning calorimetric analysis (DSC).

Fourier transform infrared spectroscopy (FTIR)

The infra-red spectra of pure moxifloxacin hydrochloride (MH) and drug-polymer physical mixture, were recorded using FTIR spectrophotometer (Perkin Elmer–spectrum Bx, USA). Disks of potassium bromide and tested sample mixtures were obtained using hydraulic press before scanning at a range of 4000 through 400 cm^{-1}.

Differential scanning calorimetric (DSC)

Unprocessed moxifloxacin hydrochloride (MH) and prepared drug-polymer physical mixture were studied regarding their thermal behavior using differential scanning calorimetry (Perkin Elmer DSC-7, USA). Aluminum pans loaded with samples equivalent to approximately 2 mg of the drug were crimped. The thermal behavior of each sample was investigated at a heating rate of 10 °C/min, covering temperature ranges of 25–200 °C. Data analysis was conducted using the TA-60WS thermal analysis software and the transition midpoint (Tm) of the drug was recorded.

Development of ophthalmic in situ gel

In situ ophthalmic gel was prepared by the hot method as per the composition of each formulation mentioned in table 1. Gelrite and benzalkonium chloride (0.0075%w/v) were dissolved in 50 ml of sterile water under an agitated condition at 1000rpm at 90 °C. After complete dissolution, tamarind gum and hydroxypropyl methylcellulose (HPMC K$_4$M) were dissolved in the same condition. The solution was kept at room temperature. Moxifloxacin hydrochloride (MH) (0.5% w/v) and mannitol (0.5 gm) were dissolved separately in water for injection separately at room temperature. The above formulation was then filtered through a polycarbonate filter of 0.45 μm, and autoclaved at 121 °C for 20 min [10-11].

Experimental design

The Box-Behnken design was used to optimize the formulation parameters and to assess the main effect and interaction effect. The independent and dependent variables are listed in table 1. Table 2, summarizes an account of the 15 experimental runs studied, their factor combinations, and the translation of the coded levels to the experimental units employed during the study. Mucoadhesion strength, gel strength, and % drug release were chosen as the response variables. Design expert software (8.0.7.1), trial version, was used for computation. Polynomial models, including interaction and quadratic terms, were generated for all the response variables using multiple linear regression analysis (MLRA) approach. The general form of the model is represented in equation 1. The goal of the experimental design was to find out, with the minimum number of experimental runs, which process variables have the biggest impact on the quality of the final product.

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2. \quad (1)$$

Where, b_0 is the intercept representing the arithmetic mean response of 15 runs, b_1 to b_{33} are the regression coefficients, X_1, X_2 and X_3 are the independent variable. The terms X_1, X_2 and X_{i2} (i=1 to 3) represent the interaction and quadratic term respectively. Box Behnken design was selected due to less number of run for 3 factors and 3 levels.

The constraint for selection of optimum formula were primarily based on the desired values of the response parameters, i.e. gel strength (<150 dyne/cm^2), mucoadhesion index (up to 15,000) and cumulative percentage drug release up to 12 h (>95%). The formulations corresponding to optimum responses were prepared and evaluated. The resultant experimental data was quantitatively compared with predicted values, and percentage error calculated [12].

Table 1: Variables and constrains in box-behnken experimental design

Independent variables	Levels			Constrains
	Low	Medium	High	
X$_1$ = Concentration of Gelrite (% W/V)	0.2	0.4	0.6	
X$_2$ = Concentration of Tamarind gum (% W/V)	0.0	0.05	1.0	
X$_3$ = Concentration of HPMC K$_4$M (%W/V)	0.0	0.25	0.5	
Transformed values	-1	0	1	
Dependent variables	Y$_1$ =Mucoadhesive index (Cps)			15,000
	Y$_2$=Gelation strength (dyne/cm^2)			<150 dyne/cm^2
	Y$_3$= Drug release up to 12 hr (%)			>95%

Data analysis and validation of the model

The statistical validity of the polynomial equation was established on the basis of ANOVA analysis. Subsequent feasibility and grid search were performed to locate design space for optimum formulations. 3-D and 2-D response surface graphs and contour plots were constructed. Checkpoint batches as per the formula were prepared and evaluated for various responses. Experimental data of all responses were quantitatively compared with that of the predicted values and validity of the model was established.

Evaluation of in situ ophthalmic gel

Clarity, pH, and viscosity

Clarity test was performed by visual inspection of each container under a good light, viewed against reflection into the eyes and viewed against a black and white background. The pH of prepared formulations was measured by a digital pH meter (Labindia–Pico$^+$, Japan). To assess the rheological property of in situ gel, the viscosity of all the prepared batches were measured at 4 shear rate i.e. 50 rpm, 100 rpm, 150 rpm, and 200 rpm using a Brookfield LVDV II PRO+viscometer. All the measurements were performed in triplicate and mean viscosity was calculated. The rheological flow behavior was determined from the graph of viscosity and shear rate [13].

Drug content

The drug content was determined for the prepared in situ gel by dissolving an amount equivalent to 50 mg of the drug from each formulation in 50 ml water. After suitable dilution, drug concentration was determined spectrophotometrically at 298 nm. The experiment was performed in triplicate.

Mucoadhesion strength

In this method mucin dispersion (MUC) 20% (w/w) was prepared by hydrating dried mucin with phosphate buffer (pH 7.4) for 12h at room temperature. Fifteen grams of this dispersion was mixed for 20 min with 5g of prepared sol before measurement that yields 15% (w/w) of mucin. The viscosity of the prepared sol/mucin system (η_t) and mucin (η_m) were measured at 32 °C at shear rates of 25, 50, 100 and 150 S^{-1}. The viscosity of the prepared sol/phosphate buffer (pH 7.4) (η_p) was determined in the same way [14]. The viscosity

component due to bioadhesion η_b was obtained by the following equation:

$$\eta_b = \eta_t - \eta_m - \eta_p \ldots \ldots (2)$$

The mucoadhesion index M [Pa] was calculated using the shear rate $D[S^{-1}]$ and the viscosity component η_b [mPas] according to the following equation:

$$M = \eta_b * D \ldots \ldots (3)$$

Gel strength

To mimic the *in vivo* conditions, in situ gel were diluted with simulated tear fluid (40:7) and transferred to a 100 ml cylinder. The gelation was performed at 37 °C. A disc (1 g in weight and 1.5 cm in diameter) was placed on the surface of the gelled solution in the cylinder, and various weights were placed on gel and gel strength was determined as the minimal weight needed for the gel to travel the disc 5 cm down. All measurements were performed in triplicate [15].

In vitro diffusion study

To ensure sustain release behavior of in situ gel, Franz diffusion cell was used. A cellulose acetate membrane (Dialysis membrane with 25 mm diameter) was adapted to the terminal portion of the cylindrical donor compartment. 3 ml of a formulation containing drug sufficient for establishing sink conditions, was placed into the donor compartment. The receptor compartment contained 15 ml of phosphate buffer solution of pH 7.4, maintained at 37 °C under mild agitation using a magnetic stirrer. At specific time intervals, aliquots of 1 ml were withdrawn and immediately restored with the same volume of fresh phosphate buffer. The amount of drug released was assessed by measuring the absorbance at 289 nm using a UV spectrophotometer [16]**.**

Ocular irritation study

To assess *in vivo* irritancy study, New Zealand Albino rabbits weighing 3.0-3.5 kg were used. They were treated as prescribed in the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 92-93, revised 1985). The preclinical experimental protocol (Protocol no. 1124 dated 8th Oct. 2011) was approved by institutional animal ethics committee as per the guidance of CPCSEA, Ministry of social justice and empowerment, Government of India (Ethical committee Registration number is 277/CPCSEA). Prior to the experiments, the animals were housed in standard cages in a light-controlled room at 19±1 °C and 50±5% relative humidity, with no restriction of food or water. During the experiments, the rabbits were placed in restraining boxes, where they could move their heads and eyes freely. All experiments were carried out under veterinary supervision. The study was performed according to Modified Draize technique on six male albino rabbits. Rabbits were divided into two groups. Group I served as control group, and Group II was treated with drug formulation. The optimized formulation was instilled daily for a period of 21 d, and the rabbits were observed for redness, swelling, and watering of the eye [17-19].

Stability study

To assess the formulation stability studies were conducted as per ICH guideline. Optimized formulation was filled in 50 ml of LDPE plastic bottle and kept in the stability chamber at 25 ̊C±5 ̊C for 3 mo. The physical and chemical stability was tested by monitoring change in pH, gelation time, mucoadhesive strength, viscosity, and drug content.

RESULTS AND DISCUSSION

Drug-polymer compatibility studies

Fourier transform infrared spectroscopy (FTIR)

The infrared study was performed to examine any possible interaction between pure drug and additives. Fig. 1 shows the FTIR spectra of moxifloxacin hydrochloride and physical mixture.

Pure moxifloxacin hydrochloride (MH) spectra showed characteristic peaks represented as C =O stretching vibrations shown at 1709 cm^{-1}, N-H stretching vibrations at 2949 cm^{-1}, O-H stretching vibration at 3530 cm^{-1}. The FTIR spectrums of physical mixture revealed the main absorption bands of moxifloxacin hydrochloride with no significant changes compared with the spectrum of pure drug. This would suggest the absence of any possible interaction between the drug and polymers.
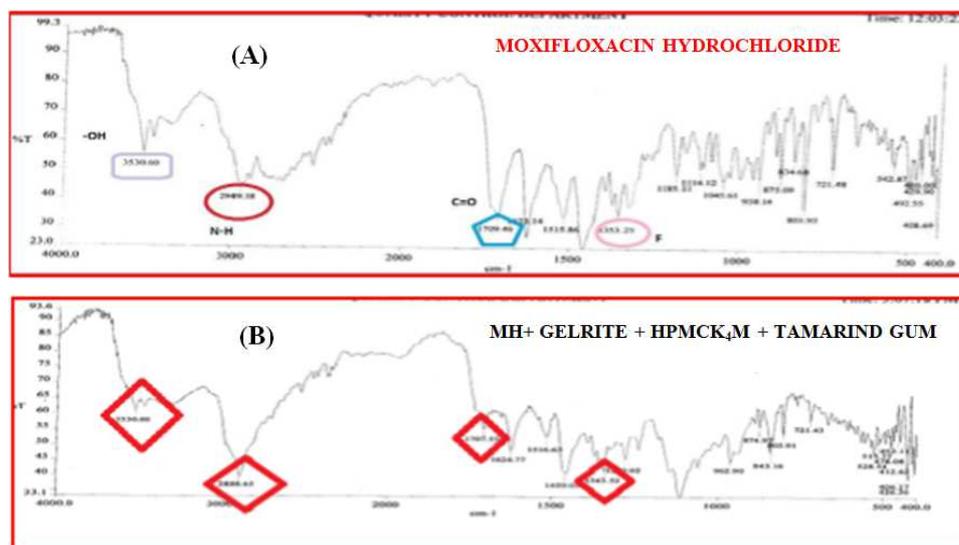

Fig. 1: FTIR spectrum of (A) Moxifloxacin hydrochloride (B) Moxifloxacin hydrochloride-polymer physical mixture

Differential scanning calorimetry (DSC)

DSC of pure moxifloxacin hydrochloride (MH) and physical mixture are shown in fig. 2. Unprocessed drug exhibited a characteristic sharp endothermic peak at 262.92 °C corresponding to its melting point. The thermo gram of the investigated physical mixture exhibited the characteristic endothermic peak of MH, indicating the absence of interaction between the two components present in the physical mixture.

Experimental design

For the response surface methodology involving BBD, a total of 15 experiments were performed for three factors at three levels each. The experiment runs with independent variables and the observed responses for the 15 formulations are shown in table 2. A suitable polynomial equation involving the individual main effects and interaction factors was selected based on the estimation of several statistical parameters, such as the multiple

correlation coefficient (r^2), adjusted multiple correlation coefficient (adjusted r^2) and the predicted residual sum of squares (PRESS), provided by the Design-Expert software® (8.0.7.1) Trial Version.
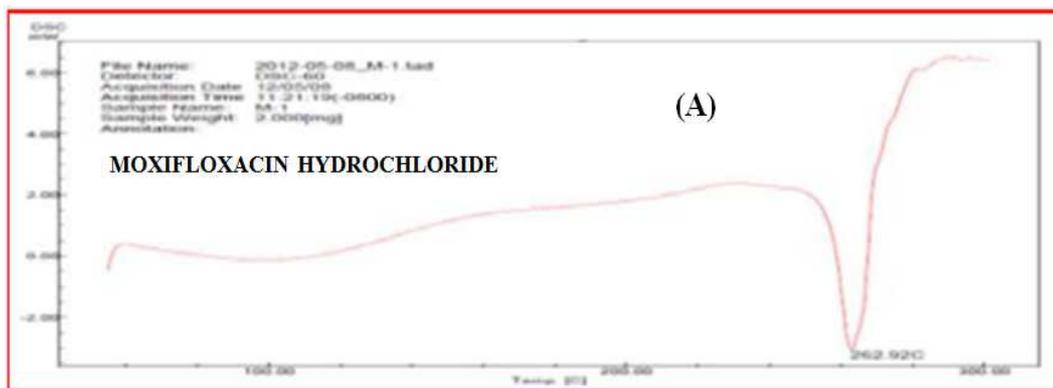

Fig. 2: DSC spectra of (A) Moxifloxacin hydrochloride (B) Moxifloxacin hydrochloride-polymer physical mixture

Table 2: Observed responses in box behnken design for in situ gel

Batch no.	Coded factor			Response		
	X_1 (Conc. of Gelrite) (%)	X_2 (Conc. of Tamarind Gum) (%)	X_3 (Conc. of HPMC K4M) (%)	Y_1 (MI) Cps	Y_2 (GS)dyne/cm^2	Y_3 (CDR at$_{12h}$)%w/w
1	0	-1	-1	630	9.86±0.02	96.62±1.21
2	-1	0	1	6675	117.73±2.21	87.54±0.21
3	-1	0	-1	3330	25.00±1.54	98.65±1.63
4	0	-1	1	1065	65.94±2.32	99.57±1.90
5	-1	1	0	4545	109.18±1.23	99.28±2.12
6	0	0	0	5295	145.68±1.21	95.45±1.36
7	0	0	0	5280	146.01±2.21	100.86±2.24
8	0	1	-1	3870	69.2±1.65	100.13±1.36
9	1	-1	0	4230	117.4±2.54	98.23±2.32
10	1	1	0	9780	98.66±2.36	84.39±2.64
11	0	0	0	5865	146.34±2.24	96.01±3.32
12	1	0	1	1023	156.13±3.21	87.66±1.23
13	-1	-1	0	1935	25.23±1.21	97.98±2.82
14	1	0	-1	1860	19.8±2.74	99.91±2.36
15	0	1	1	7095	97.66±1.24	87.19±1.26

Each value represents the mean±SD (n = 3)

Data analysis and optimization of formula

Mathematical modeling

Multiple linear regression analysis (MLRA) and analysis of variance (ANOVA) were carried out employing the Design Expert software 8.0.1.0.

to establish a relationship between the three independent variables (X_1, X_2, and X_3) and the three dependent variables (Y_1, Y_2, and Y_3) in the Box Behnken design. The results of MLR (the value of the correlation coefficient and the values of coefficients) and ANOVA (Fisher's ratio and P values) are summarized in table 3 for the three responses.

Table 3: Analysis of variance (ANOVA) for all responses

Source	Mucoadhesion index (Y_1)			Gel strength (Y_2)			Drug release at12hr (Y_3)		
	Coded coefficient	F Value	p value	Coded coefficient	F value	p value	Coded coefficient	F value	p value
Model	4779.00			146.01			95.30		
X_1	1201.87	4.443	0.029	14.36	2.759	0.028	-1.66	1.704	0.028
X_2	2178.75	10.781	0.220	19.53	5.108	0.157	-2.68	4.443	0.068
X_3	1921.88	4.444	0.068	39.20	20.571	0.073	-4.17	10.781	0.011
X_1X_2	----	0.025	0.011	-25.67	4.411	0.006	-3.78	4.444	0.068
X_1X_3	----	4.895	0.068	10.90	0.795	0.089	-0.29	0.025	0.877
X_2X_3	----	1.09	0.877	-6.91	0.319	0.413	-3.97	4.895	0.040
X_1^2	----		----	-19.70	2.396	0.596	----		----
X_2^2	----		----	-38.70	9.252	0.182	----		----
X_3^2	----		----	-46.65	13.445	0.028	----		-----
$X_1^2X_2$	----		----			0.014	----		----
r^2	0.973 (Linear)			0.967 (Quadratic)			0.929 (Quadratic)		

Response Y_1-mucoadhesion index

The high value of the correlation coefficient (0.973) indicates a good fit between the independent variables and the first dependent variable mucoadhesion index. The linear model was found to be significant as the P value is less than 0.05. This result clearly demonstrates that at least any one of the selected independent variables have a statistically significant influence on the mucoadhesion index. Conclusions can be drawn from the numerical values of the coefficients of the main effects and interaction effect. The most significant retardation effect on the mucoadhesion index was shown by the X_1 (P = 0.029) and X_1X_2. This is obvious that gelrite which is a polysaccharide increases the crosslinking and due to that mucoadhesion also increases. Factor X_3 should also be considered significant at P value is less than 0.1. The reason behind this is the polymeric nature of hydroxypropylmethylcellulose (HPMC K_4M). The contour plot for mucoadhesion index is shown in fig. 3 to facilitate understanding by the reader. The contour lines are linear in nature in the contour plot since only the main terms (X_1 and X_2) were found significant for mucoadhesion index. The equation in terms of un-coded factors is:

$Y_1 = 4779.00+1201.87 X_1+2178.75 X_2+1921.88 X_3$

Response Y_2–gel strength

High value of the correlation coefficient (0.967) indicates a good fit between the independent variables and gel strength. The quadratic model was found to be significant as the P value is less than 0.05. This result clearly demonstrates that at least any one of the selected

independent variables have a statistically significant influence on the gel strength. Conclusions can be drawn from the numerical values of the coefficients of the main effects, interaction effect, and polynomial terms. The most significant effect on gel strength was shown by, X_1 (P = 0.028) and X_1X_2 as well as some polynomial terms. From the polynomial equation, it is clear that as the concentration of gelrite increase, the gel strength increases. Factor X_3 should also be considered significant at P value is less than 0.1. The reason behind this is the polymeric nature of hydroxypropylmethylcellulose (HPMC K_4M). The contour plot for gel strength is shown in fig. 4a, 4b and 4c to facilitate understanding by the reader. The contour lines are curvilinear in nature. The equation in terms of un-coded factors is:

$Y_2 = 146.01+14.36 X_1+19.53 X_2+39.20 X_3-25.67 X_1X_2+10.90 X_1X_3-6.91 X_2X_3-19.70 X_1{}^2-38.70 X_2{}^2-46.65 X_3{}^2$

Response Y_3-drug release at 12h

High value of the correlation coefficient (0.929) indicates a good fit between the independent variables and % cumulative drug released at 12 h. The model is significant with a P value of less than 0.05. This result clearly demonstrates that at least any one of the selected independent variables have a statistically significant influence on the % drug released at 12 h. The data are shown in table 3 and fig. 5a, 5b, and 5c indicate that the concentration of gelrite and concentration of hydroxypropylmethylcellulose (HPMC K_4M) have higher drug retardation effect. The equation in terms of un-coded factors is:

$Y_3 = 95.30-1.66 X_1-2.68 X_2-4.17 X_3-3.78 X_1X_2-0.29 X_1X_3-3.97X_2X_3$
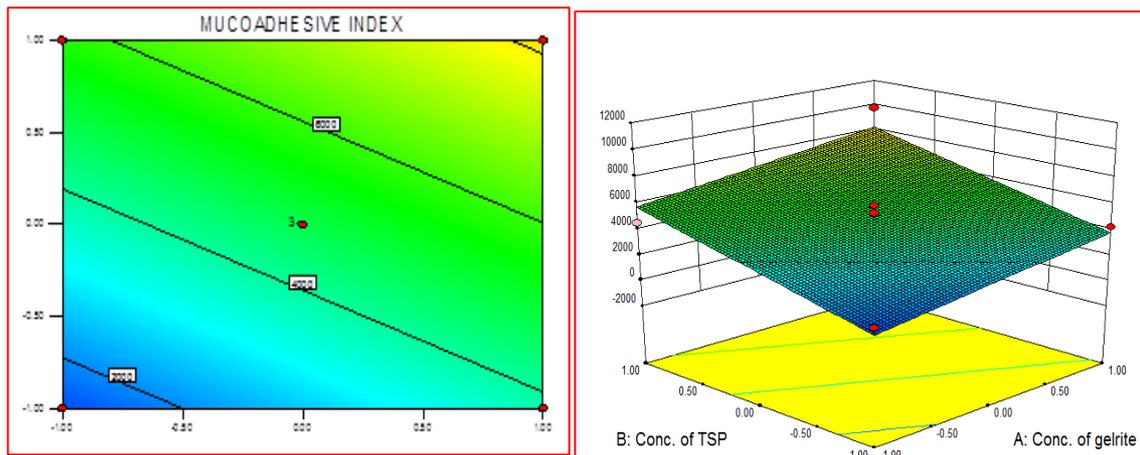

Fig. 3: Contour plot (i) and response surface (ii) plot showing the relationship between various levels of polymer (Conc. of gelrite and Conc. of tamarind Gum) on MI
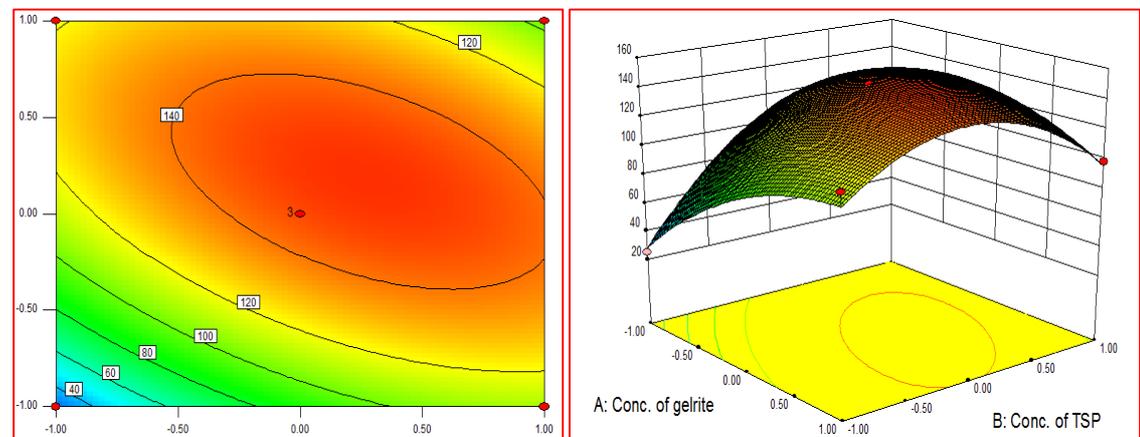

Fig. 4a: Contour plot (i) and response surface (ii) plot showing the relationship between various levels of polymer (Conc. of gelrite and conc. of tamarind gum) on GS
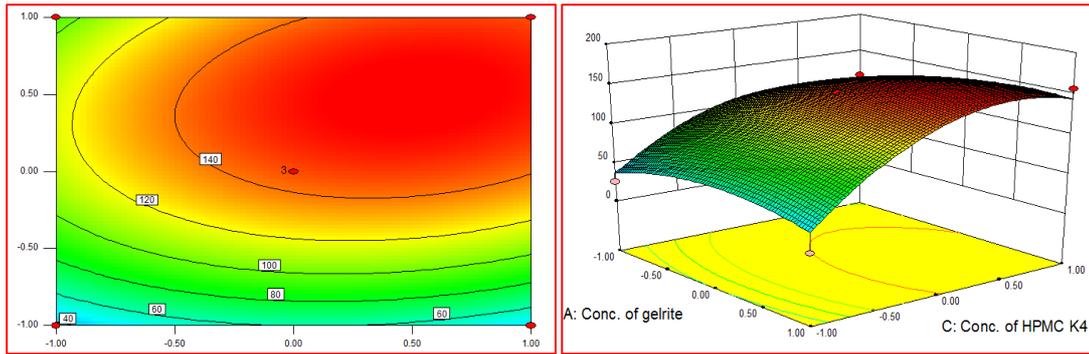
Fig. 4b: Contour plot (i) and response surface (ii) plot showing the relationship between various levels of polymer (Conc. of Gelrite and Conc. of HPMC K$_4$M) on GS
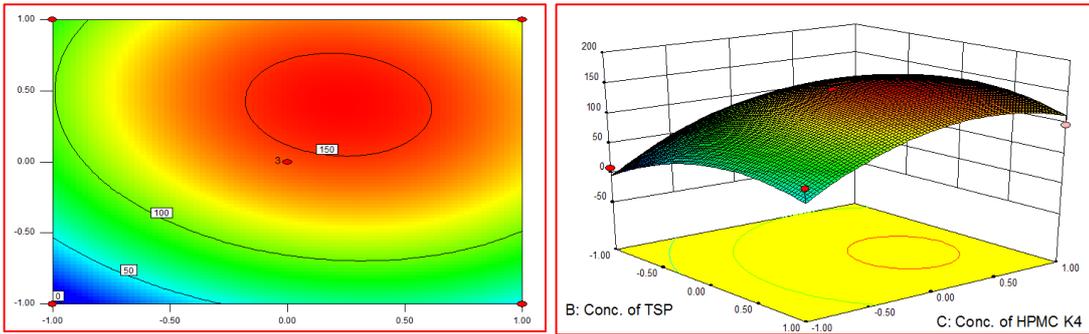

Fig. 4c: Contour plot (i) and response surface (ii) plot showing the relationship between various levels of polymer (Conc. of HPMC K$_4$M and Conc. of Tamarind Gum) on GS
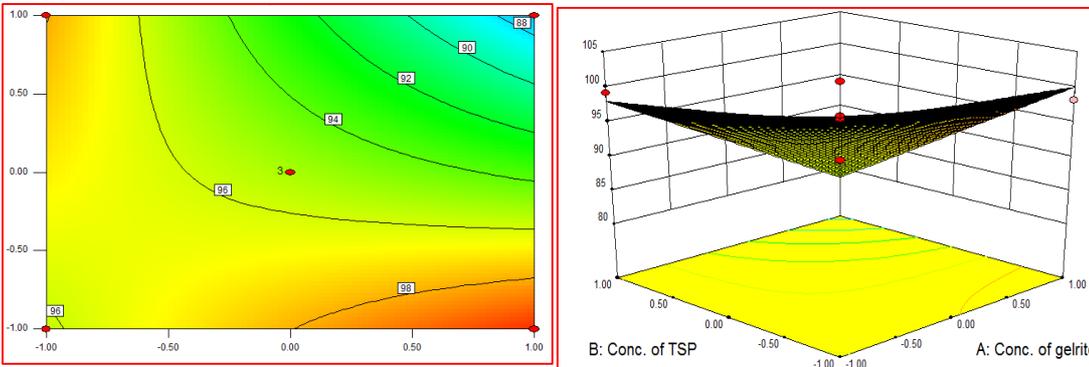

Fig. 5a: Contour plot (i) and response surface (ii) plot showing the relationship between various levels of polymer (Conc. of gelrite and conc. of tamarind gum) on drug release at$_{12h}$
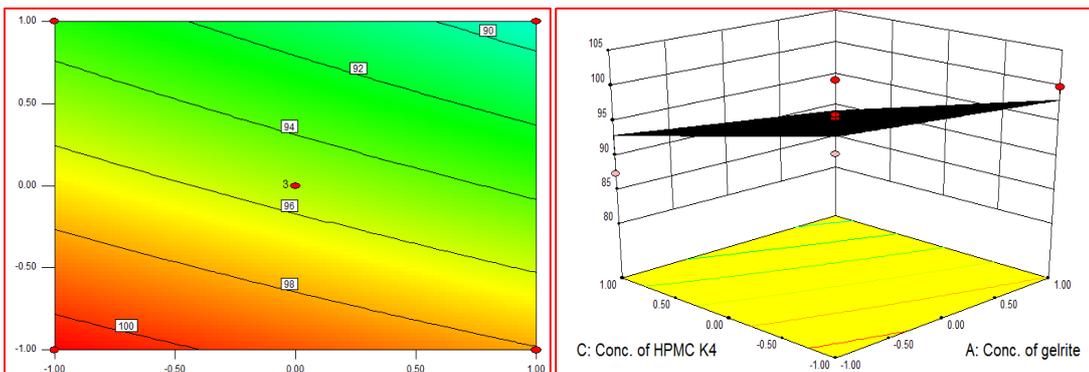

Fig. 5b: Contour plot (i) and response surface (ii) plot showing the relationship between various levels of polymer (Conc. of gelrite and conc. of HPMC K$_4$M) on drug release at$_{12h}$
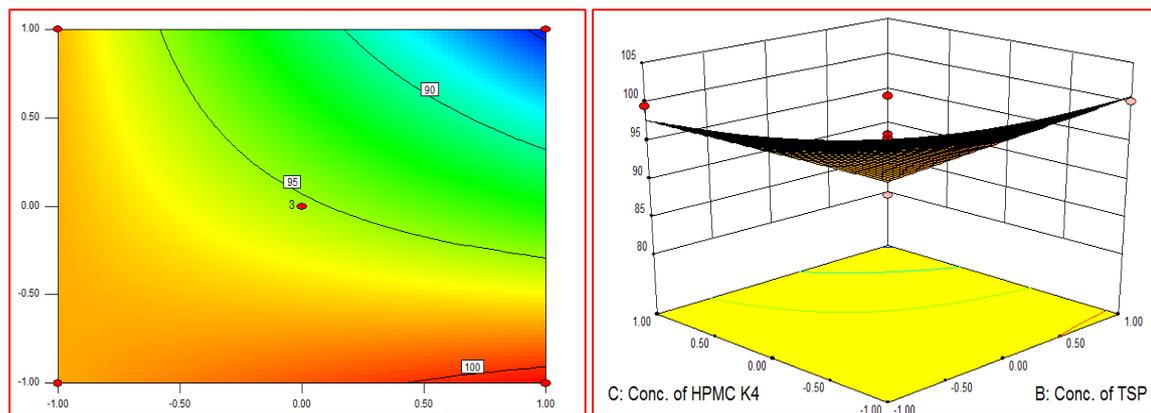
Fig. 5c: Contour plot (i) and response surface (ii) plot showing the relationship between various levels of polymer (Conc. of HPMC K₄M and conc. of tamarind Gum) on drug release at$_{12h}$

Design space and validation of response surface methodology

An ideal product is one which satisfies the requirements of mucoadhesion index, gel strength and drug release at 12h to get an idea about the acceptable. It is worthwhile to note that FDA requires that the design space be clearly defined in ANDA. Optimization was achieved by computing overall desirability. The software suggested that when the 0.6 % of gelrite, 0.023% of tamarind seed polysaccharide and 0.25 % of hydroxypropylmethylcellulose (HPMC K₄M) (see the square within the overlaid plot), the three requirements.

To check the reliability of the evolved mathematical models, responses were checked through additional random checkpoint batches covering the entire range of the experimental domain. By the use of grid search analysis, two batches were selected and responses were predicted by the mathematical model. These two additional batches were prepared, and actual responses were recorded. Table 4 shows the composition of check-point formulations, predicted and experimental values of responses and percentage predicted error. Percentage prediction error is helpful in establishing the validity of generated equations and to describe the domain of applicability of the RSM model. The prediction error was found to vary between 1.08 to 3.65. The low magnitudes of error, as well as the significant values of R2 in the present study, prove the high predictive ability of RSM.

Table 4: Composition of checkpoint batch formulation predicted the experimental value of the response variable

Check point batches	M*	M**	M***
X_1(%)	0.6	0.6	0.6
X_2(%)	0.023	0.035	0.046
X_3(%)	0.25	0.21	0.205
The predicted value of response Y_1(Cps)	5222.38	5441.24	5072.02
Actual value of response Y_1(Cps)	5380	5500	5172
% Prediction Error	3.01	1.08	1.97
Predicted value of response Y_2(dyne/cm^2)	137.06	141.49	136.99
Actual value of response Y_2 (dyne/cm^2)	141	145.65	142
% Prediction Error	2.91	2.94	3.65
Predicted value of response Y_3(%w/w)	98.15	97.37	98.09
Actual value of response Y_3(%w/w)	99.46	99.36	96.02
% Prediction Error	1.31	2.04	2.11

#predicted error (%) = (observed value-predicted value)/predicted value×100 %., M* Optimized Batch, M* and M** Check point batches having desirability near 0.99 from grid search

Evaluation of in situ gel

Clarity, pH, viscosity

Clarity of all formulations was found satisfactory. The formulations were light yellow in color. Terminal sterilization with autoclaving had no effect on physicochemical properties of the formulation. The pH was within the acceptable range (6.8 to 7.4). The successful use of in situ gels for ocular delivery is not only dependent on properties after administration but is also important that they are easy to administer, by dropping, into the eye. It should exhibit low viscosity for reproducible dose administration. Gelrite containing formulations were mixed with STF in 1:1 dilution showed the drastic difference in viscosity confirming phase transitions from sol to gel as gelrite is a cationic sensitive polymer. In this condition, formulations possessed Non-Newtonian flow, i.e., by increasing shear stress, shear thinning of the formulation was observed. This increasing shear rate mimics ocular shear rates associated with normal blinking which is extremely wide, ranging from 0.03-28500 S-1. Viscosities of prepared formulations were found in a range of 10 to 150 cps and exhibited Non-Newtonian flow [20-21].

Drug content

Drug content of the prepared in situ gel was in the range of 98-99% w/w, excluding any possibility for segregation of drug or additives during preparation.

Mucoadhesive index (MI)

The mucoadhesive index is an important physicochemical parameter for in situ forming ophthalmic gels since it prevents the formulation from rapid drainage and hence lengthens its precorneal residence time. It shows adhesive forces between polymer molecule and mucous membrane. Results of the determination of mucoadhesive forces of all the prepared formulations are tabulated in table 2 [22].

Gelation strength (GS)

Gel strength shows cohesive forces between polymer molecules. All formulations exhibited good gel strength. Results of gel strength are presented in table 2 [23].

Ocular irritation test

The optimized batch M* was selected for the ocular irritation test. The formulation for instilled daily for 21 d and was found to be non-irritating with no ocular damage to the cornea, iris or conjunctivae and moreover, redness and swelling was also not observed in the experimental animals (fig. 6).

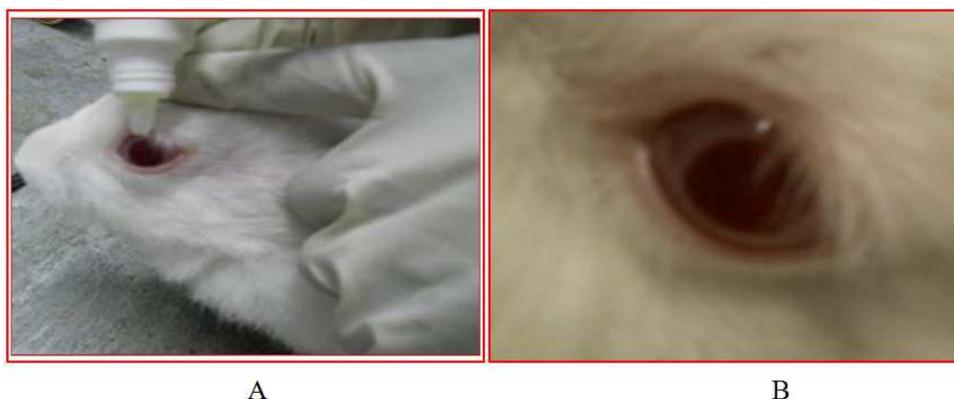Thus, batch M* was considered as therapeutically safe, efficacious and suitable for the ocular delivery of the drug [9]**.**

Fig. 6: Ocular irritation test (A) rabbit eye before ocular irritation test (B) Rabbit eye after ocular irritation test

Stability studies

Stability study of optimized formulation was performed as per ICH guideline. The sample was evaluated for *in vivo* release study, gel strength, mucoadhesive strength, and viscosity. Variation in value was found within±3% of initial value. This study shows that no change or degradation was found in moxifloxacin hydrochloride in situ gel (table 5).

Table 5: Stability study data of optimized formulation

Parameter	5 d	After 15 d	After 30 d
Appearance	Acceptable	Acceptable	Acceptable
Viscosity	48.01±0.15	48.22±0.14	49.32±0.32
pH	7.06±0.11	7.10±0.10	7.26±0.11

Each value represents the mean±SD (n = 3)

CONCLUSION

In this study, moxifloxacin hydrochloride (MH) in situ gel was successfully prepared using gelrite, hydroxypropylmethylcellulose (HPMC K$_4$M) and tamarind gum. However, preclinical and clinical studies are necessary for commercialization of dosage form. In situ ophthalmic gel was optimized using Box Behnken design. The influence of quantitative factors was predicted by a polynomial equation. Based on optimization, it was concluded that batch containing 0.6 % gelrite, 0.023 % tamarind gum and 0.25 % hydroxypropylmethylcellulose (HPMC K$_4$M) gave maximum residence time and better efficacy. On the basis of *in vivo* ocular irritancy study, it was found that optimized formulation was nonirritant. Thus, this dosage form might be a promising delivery system of ophthalmic drugs with increased bioavailability.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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