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Original Article

FORMULATION AND OPTIMIZATION OF THERMOSENSITIVE *IN-SITU* GEL OF MOXIFLOXACIN HYDROCHLORIDE FOR OCULAR DRUG DELIVERY

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ABSTRACT

Objective: The present study has focused on development and optimization of thermosensitive in-situ ocular drug delivery system for the treatment of conjunctivitis.

Methods: Thermosensitive in-situ hydrogel formulation of moxifloxacin hydrochloride was developed by dispensing variable concentration of pluronic *F-127*, gellan-gum, and carbopol in distilled water. Viscosity, gelation temperature and mean release time (MRT) were measured by using 'Brookfield' viscometer LV-III (spindle no. 40), rheological techniques and dissolution apparatus respectively. Optimization for ideal formulation was carried by 'Box–Behnken' design on the basis of prime factors of the formulation including viscosity, gelation temperature, and MRT. Moreover, the optimized formulation was evaluated for accelerated stability study by *in vitro* drug release, anti-microbial potential by 'Kirby-Bauer disk diffusion' method and ocular irritancy assay were done by *in vivo* analysis.

Results: Optimised thermosensitive in-situ gel, when administered into cul-de-sac region of the eye, it was immediately transformed from sol to gel by multi-dimensional mechanism due to plurionic, gellan-gum, carbopol. The optimized formulation minimizes the chances of formulation failure as well as the concentration on individual polymer which dependence on a single mechanism of gelation. The final optimised formulation consists of plurionic (11.50% w/v), gellan-gum (0.32% w/v), carbopol (0.3% w/v), shows optimum therapeutic effect. Moreover, the accelerated stability study, anti-microbial potential, and ocular irritancy confirmed the biocompatibility of optimized *in-situ* drug-containing gel with high potency and stability.

Conclusion: Thus, optimized *in-situ* drug-containing gel with multifactorial approaches showed promising ocular formulation having minimum side effect and high therapeutic efficacy.

Keywords: Ocular *in-situ* gel, Pluronic *F-127*, Gellan-gum, Carbopol, Box–Behnken design, Bioavailability

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INTRODUCTION

In-situ drug (gel) delivery systems concern with the conversion of the liquid state of a formulation (solution) into the gel at the site of the application under specific physiological conditions. Numerous factors regulate the formation of in-situ gel including temperature, pH, solvent exchange, ionic cross-linkage and ultraviolet-irradiation [1]. Since, these approaches do not need any external stimulation, co-polymerizing agent or organic solvents, thus attracts great attention of researcher [2, 3]. In-situ drug delivery systems can significantly reduce the concentration and frequency of daily dose, improve the biocompatibility, patient compliance, and cost-effectiveness. Indeed, some earlier approaches have been made to enhance the ocular drug bioavailability by enhancing the precorneal residence time, as the drug may undergo several phases for transformation from sol to gel after administration into cul-de-sac eye region [4].

Although, many ophthalmic in-situ gel preparations were reported in previous investigations, that mainly based on the principle of pH sensitivity (cellulose acetophthalate) [5, 6], temperature regulation (copolymers of poly (oxyethylene) and poly (oxypolylene)) [7, 8] and ionic formation (alginate and Gelrite) [9, 10]. But the approaches have been focused on the individual mechanism (pH dependent, temperature dependent or ionic dependent), made the final formulation become bulkier due to the high concentration of polymers. Additionally, the formulation becomes more expensive for an instance, if pluronic is considered as the main polymer, then we need about 30% concentration of this polymer for achieving sufficient gelling strength of formulation which is very high. In case of carbopol, the overall pH of the formulation becomes reduced due to self-acidic nature of carbopol and which may cause excessive tearing and washing of formulation [11]. Furthermore, in the case of gellan gum, more time is needed for gel formation, which leads to failure of the formulation to convert from sol into the gel before being washed away. Moreover, the formulation was not much success, as the individual polymer has its own limitations at high concentration including eye irritation (carbopol), thermosensitive (pluronic) and low rate of gelation at the site of application (gellan-gum). Whereas, combining multiple polymers, we can formulate a robust formulation which reduces the chances of formulation failure and reduces the concentration of polymers required for the formulation of in-situ gel [4, 12]. Flouroquinolones are considered as the main drug for the treatment of *pseudomonas aeruginosa* infection [13, 14]. Moxifloxacin hydrochloride is a fluoroquinolone antibiotic which is generally used to treat the various bacterial infection such as conjunctivitis, pneumonia, endocarditis, sinusitis, and tuberculosis. Previously sol-to-gel system of moxifloxacin hydrochloride was prepared using carbopol, gellan-gum, and pluronic polymers individually. Thus, to overcome the limitation of the conventional formulation including rapid elimination, poor ocular bioavailability and eye irritation we have proposed the advanced formulation of moxifloxacin hydrochloride based on multifactorial approaches. We have targeted the combination of multiple mechanisms to form the new thermo sensitive formulation having a comparatively low concentration of each polymer. The various combinations of polymers of investigated formulations have been optimized by design expert software (Design-Expert® 10 software) for maximum drug release, suitable viscosity at the ocular site and optimized gelation temperature. Furthermore, the ocular irritancy was observed in rabbit's eye for 14 d.

MATERIALS AND METHODS

Material

Moxifloxacin hydrochloride was obtained as a gift sample from INTAS Pharmaceuticals, Ahmadabad, India. pluronic F-127, carbopol 934, gellan-gum were obtained as a gift sample from Jubilant Life Sciences India. All other chemicals were of analytical grade.

Microbial culture *pseudomonas aueroginosa* and *staphylococcus aureus* were obtained from microbial type culture collection (MTCC); MTCC catalogue no. of both are 424 and 96 respectively, and Gene Bank, CSIR-Institute of Microbial Technology, India.

Methods

Preparation of in-situ hydrogel and drug loading

In-situ hydrogel system was prepared by dispersing pluronic F-127 (10-20%) into distilled water with continues stirring for 1 hr. For the preparation of the gellan-gum solution, gellan-gum (0.1-0.5%) was dispersed into distilled water heated to 90 °C and then allowing to cool at room temperature with continuous stirring for 1hr. Carbopol (0.1-0.5) was dispersed into distilled water. Partially dissolved solutions of polymer were kept in a refrigerator overnight at 4 °C, until the entire polymer had dissolved. The in-situ hydrogel was prepared by mixing of the specified amount of pluronic F-127, gellan-gum and carbopol under continuous stirring for 1hr followed by over-night refrigeration.

For the preparation of moxifloxacin hydrochloride containing in-situ gel, the solution of moxifloxacin hydrochloride was dispersed into a solution of pluronic, gellan-gum and carbopol with continuous stirring for 5 min followed by addition of benzalkonium chloride (preservative) solution. The pH of samples was adjusted at 7-7.5 by addition of 1 M sodium hydroxide solution. Finally, the samples were sterilized at 121 °C at 15 psi for 30 min and the formulation was refrigerated at 4 °C. Variables of the formulation were used in the development of in-situ formulation as described in table 1 and the compositions of in-situ hydrogel formulation have described in table 2.

Table 1: Variables used in the formulation of thermosensitive *in-situ* hydrogel system

S. No.	Variables	Levels		
		I	II	III
1	Pluronic *F-127*	10	15	20
2	Gellan-gum	0.1	0.3	0.5
3	Carbopol	0.1	0.3	0.5

Table 2: Formulation composition of the *in-situ* hydrogel

S. No.	Ingredients	Quantity (g)
1	Moxifloxacin hydrochloride	0.3
2	Pluronic *F-127*	10-20
3	Gellan-gum	0.1-0.5
4	Carbopol	0.1-0.5
5	Benzalkonium Chloride	0.006
6	Distilled Water	Quantity sufficient for 100 g

Physical parameters

The prepared *in-situ* hydrogel was evaluated for physical properties such as appearance, colour and texture etc.

Rheological studies

The rheological study was carried out by using Brookfield Viscometer LV-III. Briefly, 100 gm of the sample was taken in a beaker of 100 ml and viscosity was measured by using spindle no. 40.

***In vitro* release study**

In vitro release studies of the prepared formulation were done with the help of modified dissolution apparatus. An overnight soaked semi-permeable membrane was tied to one end of an open-ended cylindrical tube having the diameter of 3.2 cm. 2 ml of the test sample was placed into the dissolution apparatus using simulated tear (pH 7.4) as dissolution medium. Finally, the apparatus was suspended into the beaker containing 100 ml of dissolution medium, maintained at 37±2 °C and 50 rpm (by magnetic stirrer). Periodically 1 ml of sample was withdrawn and replaced by fresh dissolution medium. The aliquots were analysed at 287 nm using ultraviolet spectrophotometry.

Mean release time (MRT)

MRT was determined by using equation.

$$MRT = \frac{\sum_1^t tmid^{\Delta C}}{\sum_1^t \Delta C}$$

In the above equation, the european journal of pharmaceutical represents the time at the mid-point between *i* and *i-l* and ΔC represents the additional concentration of drug release between *i* and *i-l* [15].

Measurement of gelation temperature

For the measurement of gelation temperature, 10 ml sample was taken and placed in 20 ml beaker. A magnetic stirring bar was placed in the beaker. The temperature of the sample was increased gradually at the rate of 1 ˚C/min and the temperature at which the movement of a magnetic bar was hindered was recorded as the gelation temperature [16, 17].

Design of experiment

Experiment designing of the *in-situ* gel of moxifloxacin hydrochloride was primarily screened by trailed performed using variable factors like pluronic *F-127*, gellan-gum and carbopol. These trials-based screening were recognised as a significant parameter in formulation design for desirable drug delivery and formulation characteristics.

We used Box-Behnken design (Design-Expert® 10 software) for the evaluation of formulation variables on product characteristics. About 13 runs (n=13) of the experiment were designed with 1 centre point having randomised 200 gm batch sizes. The variable factors and the response data are as shown in table 1 and 2.

Antimicrobial activity

Antimicrobial activity was performed to evaluate the efficiency of the optimized formulation as compared to the marketed formulation. Antimicrobial activity Kirby-Bauer disk diffusion method was used where the drug-loaded disk of 10 mm diameter was incubated in nutrient agar media. Nutrient media were pre-poured into the sterile petri plates and allowed to cool under laminar air flow. Microbial culture *pseudomonas aueroginosa* and *staphylococcus aureus* were inoculated with the help of sterile cotton swabs and drug-loaded disks were placed in the inoculated media. Finally, the petri plates were incubated at 37±0.5 °C for 24 hr. Zone of inhibition was measured with the help of vernier calliper [18].

Accelerated stability study

Optimized formulation of moxifloxacin hydrochloride was tested for accelerated stability study. In brief, the optimised formulation was placed in glass vial closed by the grey butyl rubber closure and sealed with aluminium closure was stored in a stability study

chamber at 40±2 °C, 75±5% RH in both horizontal and vertical position for 1 mo. Periodically samples were withdrawn and evaluated for change in visual appearance, pH, gelling capacity, drug content and *in vitro* drug release [19].

Ocular irritancy

Ocular irritancy test was done on rabbits eye (either sex), a weight of 2-3 kg obtained from the institutional animal house (Amity University) by protocol approval number: CPCSEA/IAEC/AIP/ 2017/08/16. Experimental animals were acclimatised for 4 d before starting the experimental work. 0.1 ml of the optimized formulation of moxifloxacin hydrochloride was used twice a day for administration into the cul-de-sac for the duration of 14 d. The animals were evaluated for the watering of eyes, mucosal discharge and swelling.

RESULTS AND DISCUSSION

Physical evaluation

The prepared in-situ gel was clear, translucent, non-gritty, uniform in nature.

Rheological evaluation

Rheological evaluation of the formulation is done to evaluate the viscosity of the formulation. The rheological study of the samples is done. The viscosity of the prepared formulation was measured for formulation 1 to 13 was 295, 250, 485, 255, 210, 225, 460, 475, 265, 307, 235, 280 and 460 mPa. S consecutively (table 3).

Evaluation of MRT

MRT has been taken by the formulation to release it's 80% of drug content. MRT of the prepared formulation was evaluated. The MRT of the formulations 1 to 13 was measured at 2.9, 2.8, 3.1, 2.7, 2.6, 2.6, 3.1, 2.9, 2.9, 3.1, 2.8, 2.9 and 2.9 hr. respectively (table 3).

Gelation temperature

Gelation temperature of the formulation indicates the temperature at which the phase conversion of formulation has been taken place from liquid to solid. The gelation temperature of the prepared formulations from 1 to 13 was found to be 34, 27, 17, 36, 29, 36, 24, 33, 20, 18, 20, 27 and 26 respectively (table 3).

Table 3: Composition of various factors used in thermosensitive *in-situ* ocular drug delivery gel of moxifloxacin hydrochloride and responses

Run	Factors			Responses		
	Pluronic *F-127*	Gellan-gum	Carbopol	MRT (h)	Gelation temperature (°C)	Viscosity (mPa. S)
1	10	0.3	0.5	2.9	34	295
2	15	0.1	0.5	2.8	27	250
3	20	0.5	0.3	3.1	17	485
4	10	0.3	0.1	2.7	36	255
5	15	0.1	0.1	2.6	29	210
6	10	0.1	0.3	2.6	36	225
7	15	0.5	0.5	3.1	24	460
8	10	0.5	0.3	2.9	33	475
9	20	0.3	0.1	2.9	20	265
10	20	0.3	0.5	3.1	18	307
11	20	0.1	0.3	2.8	20	235
12	15	0.3	0.3	2.9	27	280
13	15	0.5	0.1	2.9	26	460

Table 4: Fit summery of response 1, 2 and 3. df represents the degree of freedom; PRESS represents predicted residual error sum of squares; statistically significant are underlined (*p*-value<0.005)

Source	Sum of squares			df			*F* value			Prob>*F* *p*-value		
	y_1	y_2	y_3	y_1	y_2	y_3	y_1	y_2	y_3	y_1	y_2	y_3
Sequential sum of model square												
Linear	0.34	538.00	1.495E+005	3	3	3	132.60	2098.20	28.16	<0.0001	<0.0001	<0.0001
2FI	0.000	0.000	401.00	3	3	3	0.000	0.000	0.052	0.0000	1.0000	0.9830
Quadratic	7.692E-003	0.77	15105.31	3	3	3	-	-	35.88	-	-	0.0075
Cubic	0.000	0.000	421.00	3	3	3	-	-	-	-	-	-
Residual	0.000	0.000	0.000	0	0	0	-	-	-	-	-	-
Total	107.37	9801.00	1.976E+006	13	13	13	-	-	-	-	-	-
Source	R-squared			Adjusted R-squared			Predicted R-squared			PRESS		
	y_1	y_2	y_3	y_1	y_2	y3	y_1	y_2	y_3	y_1	y_2	y_3
Model summary statistics												
Linear	0.9779	0.9986	0.9037	0.9705	0.9981	0.8716	0.9640	0.9970	0.7991	0.016	1.60	33225.15
2FI	0.9779	0.9986	0.9061	0.9558	0.9971	0.8123	0.8884	0.9928	0.5255	0.039	3.88	78494.25
Quadratic	1.000	1.0000	0.9975	1.0000	1.0000	0.9898	-	-	-	+	+	+
Cubic	-	-	-	-	0.9927	-	-	-	-	+	+	+

Details of ANOVA of response y1, y2 and y3 have described in table 5, also details of the value of multiple regression terms were analysed and presented in table 6.

Optimization of formulation

Independent variables including pluronic F-127, carbopol and gellan-gum were evaluated for their final effect on MRT, gelation temperature and viscosity. Total 13 experiments were conducted and the response data were generated in according to Box-Behnken design (table 3).

Final data obtained from the experiments were well modelled by the independent variable linear function. Hence first order polynomial equation was used for approximating the function.

$y = \beta 0+\beta 1 \chi 1+\beta 2 \chi 2+\beta 3 \chi 3+€. .$ (1)

'€' represents the noise or error,

'χ' represent independent variable,

'y' represents response and

'β' represents the coefficient.

The value of responses y1 (MRT), y2 (gelation temperature) and y3 (viscosity) varies from 2.6 to 3.1 hr, 17 to 36 °C, and 210 to 485 mPa. S. The ratio of maximum to minimum response for response y1, y2, and y3 was 1.19, 2.11, and 2.3 respectively. Thus, power transformation was not applicable to the obtained values. The model section for response analyzing was according to the sequence model sum of square, lack of fit test and model summary statistics. The Prob>F value of p<0.0001, low standard deviation, high R-square value and lower predicted residual error sum of square (PRESS) value recommend to select the linear model for analysing the responses as detailed in table 4.

Analysis of variance (ANOVA) was applicable to determine the effect of many variables and their interaction. The regression model was used to develop contour plots of independent factors. The ANOVA table affirms the competence of the liner model (Model Prob>F is less than 0.05). It also determines the powerful factors that affect the response y1, y2 and y3 of the formulation. For MRT, the concentration of gellan-gum played a significant role, followed by other 2 factors.

Moreover, for gelation temperature, the concentration of polymer pluronic was determined as the significant model term, whereas for viscosity concentration of gellan-gum was found to be significant as compared to the recent investigation [20].

Table 5: ANOVA response for a surface model of MRT, gelation temperature and viscosity

Source	Sum of squares			df			Fvalue			Prob>F P-value		
	y_1	y_2	y_3	y_1	y_2	y_3	y_1	y_2	y_3	y_1	y_2	y_3
Model	0.34	538.00	1.650E+005	3	3	9	132.60	2098.20	130.63	<0.0001	<0.0001	0.0010
A	0.080	512.00	220.50	1	1	1	93.60	5990.40	1.57	<0.0001	<0.0001	0.2988
B	0.18	18.00	1.152E+005	1	1	1	210.60	210.60	820.90	<0.0001	<0.0001	<0.0001
C	0.080	8.00	34060.50	1	1	1	93.60	93.60	242.71	<0.0001	<0.0001	0.0006
Residual	7.692E-003	0.77	421.00	9	9	3	-	-	-	-	-	-
Cor total	0.35	538.77	1.654E+005	12	12	12	-	-	-	-	-	-

Table 6: Value of regression term

S. No.	Terms	Values		
		y_1	y_2	y_3
1	R-squared	0.9779	0.9986	0.9975
2	Adj R-squared	0.9705	0.9981	0.9898
3	Pred R-squared	0.9540	0.9970	-
4	Adeq precision	30.832	117.162	35.660

The "Pred R-squared" value of response was found to be in reasonable agreement with the "Adj R-squared" value which indicates that the model has predicted the response value significantly.

The eventual mathematical model determined by software design-expert was demonstrated in Equation 2, 3 and 4.

y1 (MRT) =+2.194+0.020 ∗ A+0.750 ∗ B+0.50∗ C. . (2)

y2 (Gelation temp) =+54.442–1.600∗ A–7.500∗ B–5.000∗ C. . (3)

y3 (Viscosity) =+234.625–5.400∗ A–371.250∗ B+215.00∗ C. . (4)

The positive sign represents a synergistic effect, whereas a negative sign represents an antagonistic effect.

In case of the y1 positive coefficient of A in the model refers to increase in MRT at higher concentration of pluronic F-127. Similarly, the positive coefficient of B and C indicated the increase in MRT with increasing other factors (pluronic, carbopol and gellan-gum concentration). For y2, negative coefficient of A, B, C referred to decrease in gelation temperature as an increase in the concentration of other factors. Whereas for the y3 negative coefficient of A and B referred to decrease in viscosity as the concentration of these factors increases, and positive coefficient represented the increase in the viscosity with an increase in the concentration of factor C.

Theoretical and experimental response values were compared with each other using diagnostic case statistical report showed (table 7) reasonably close agreement.

Table 7: Actual and predicted value of the responses, statistically significant terms are underlined (*P*-value less than 0.005)

Std. order	Actual value			Predicted value			Residual value		
	y_1 (h)	y_2 (°C)	y_3(m. Pa. S)	y_1(h)	y_2(°C)	y_3(m. Pa. S)	y_1(h)	y_2(°C)	y_3(m. Pa. S)
1	2.90	34.00	395.00	2.87	33.69	390.00	0.031	0.31	5.00
2	2.80	27.00	350.00	2.82	27.19	350.25	-0.019	-0.19	-0.25
3	3.10	17.00	535.00	3.12	17.19	530.25	-0.019	-0.19	4.75
4	2.70	36.00	255.00	2.67	35.69	260.50	0.031	0.31	-5.50
5	2.60	29.00	210.00	2.62	29.19	199.75	-0.019	-0.19	10.25
6	2.60	36.00	275.00	2.62	36.19	279.75	-0.019	-0.19	-4.75
7	3.10	24.00	560.00	3.12	24.19	570.25	-0.019	-0.19	-10.25
8	2.90	33.00	525.00	2.92	33.19	519.75	-0.019	-0.19	5.25
9	2.90	20.00	265.00	2.87	19.69	270.00	0.031	0.31	-5.00
10	3.10	18.00	407.00	3.07	17.69	401.50	0.031	0.31	5.50
11	2.80	20.00	285.00	2.82	20.19	290.25	-0.019	-0.19	-5.25
12	2.90	27.00	330.00	2.87	26.69	330.00	0.031	0.31	0.000
13	2.90	26.00	460.00	2.92	26.19	459.75	-0.019	-0.19	0.25

Perturbation graph was plotted to determine the factors which mostly affect the response. A high slope or curvature in perturbation graph indicates sensitivity against specific factor whereas, a relatively flat line indicates the less sensitivity towards that factor. In case of response y1 (MRT) factor B showed high slope that indicates higher sensitivity towards factor C, whereas factor A and C possesses relatively less slope indicating lesser sensitivity for response y1 (fig. 1). For response y2, factor A showed a steep slope and factor B, and C shows slight slop indicating a limited effect on response y2 (fig. 2). Moreover, for response y3 factor B and C represented a significant relation, whereas factor A showed very less slope than factor B and C, indicating the lesser effect on response y3 (fig. 3).

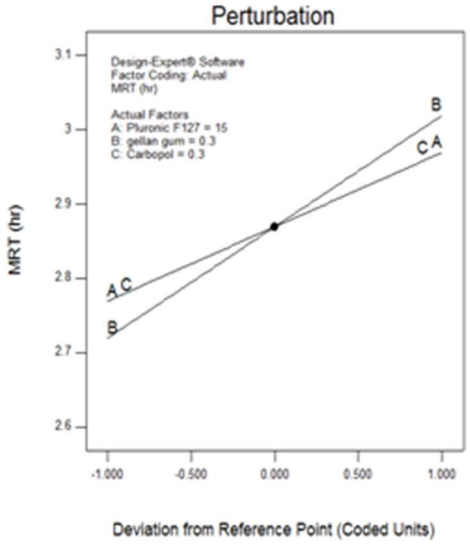

Fig. 1: Perturbation graph for effect of individual factor on response y1(MRT)

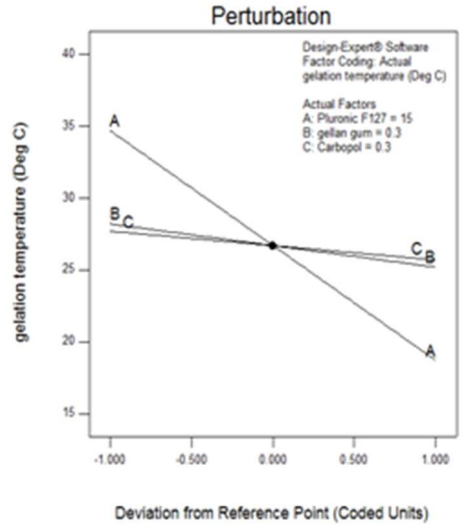

Fig. 2: Perturbation graph for effect of individual factor on response y2 gelation temperature

By using contour plots the relationship between independent and dependent variable was elucidated. Fig. 4 to 6 represents the relationship of factors A, B and C on the responses y1, y2 and y3.

Fig. 4 shows the effect of factor A and B on response y1 while keeping other factors such C at constant levels. The graph indicates the direct relation of concentration of factor A and B with response y1 (MRT). Increase in the concentration of factor A and B, showed the increase in MRT of the formulation. Fig. 5 shows the effect of A and C on response y1 while keeping other factors (B) at constant levels.

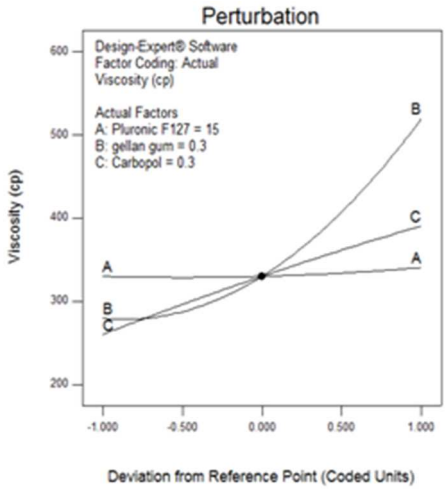

Fig. 3: Perturbation graph for effect of individual factor on response y3 viscosity

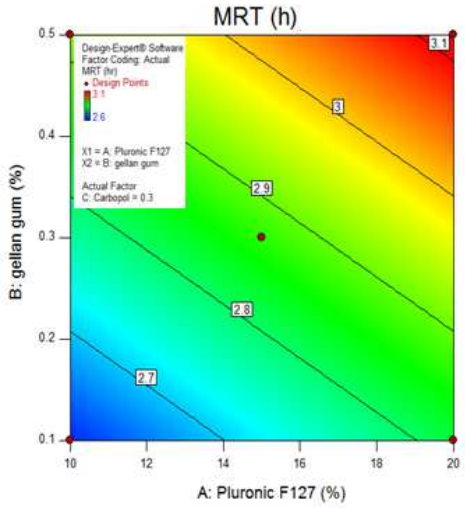

Fig. 4: Contour plot of the effect of pluronic *F-127* (A) and gellan-gum (B) on MRT

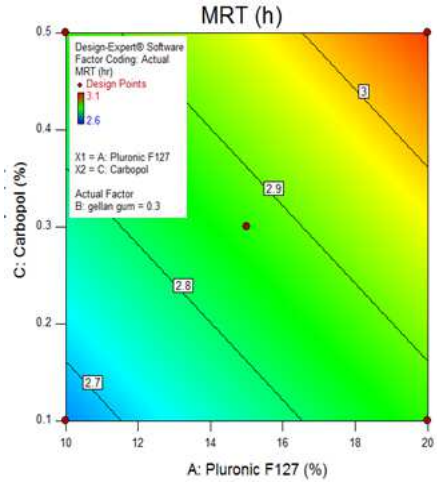

Fig. 5: Contour plot of the effect of pluronic *F-127* (A) and carbopol (C) on MRT

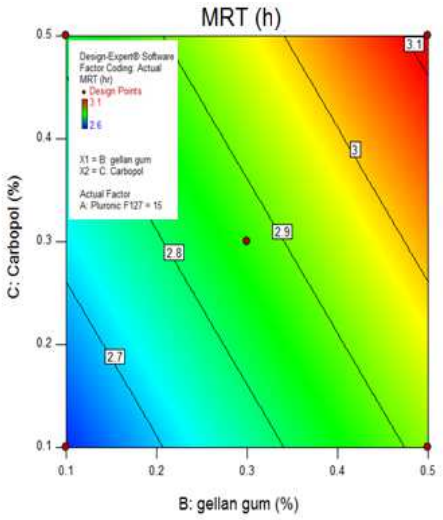

Fig. 6: Contour plot of the effect of gellan-gum (B) and carbopol (C) on MRT

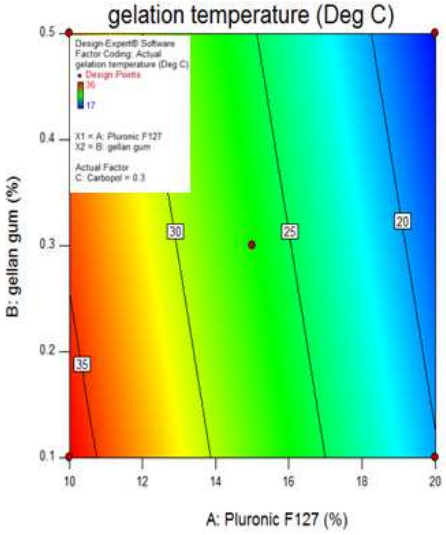

Fig. 7: Contour plot of the effect of pluronic *F-127* (A) and gellan-gum (B) on gelation temperature

The graph indicates the direct relation of response y1 (MRT) towards the concentration of the factor A and C. Similarly, fig. 6 shows the effect of factor B and C on response y1, while keeping another factor A at a constant level. The above graph indicates the direct relationship of the factor B and C, with response y1 (MRT).

Fig. 7 shows the effect of factor A and B on response y2. While keeping other factor C at the constant level. The above graph indicates the negative relation of factor A and B which revealed that the increase in the concentration of either of factors, the response y2 (gelation temperature) will decrease, but factor B has less impact on this response as compared to factor A. Fig. 8 shows the effect of factor A and C on response y2 while keeping other factor B at a constant level. The graph also indicates the negative relation of factors A and C with response y2, as the increase in either of these factors responsible to decrease the response y2. Whereas, factor C having the less significant impact towards response y2. Fig. 9 shows the effect of factor B and C on response y2 while keeping factor A at a constant level. Moreover, the graph indicates the negative relation of factors B and C with response y2 as the increase in factor B and C concentration significantly reduced response y2 (gelation temperature). Both of these factors were showing equal significance toward response y2.

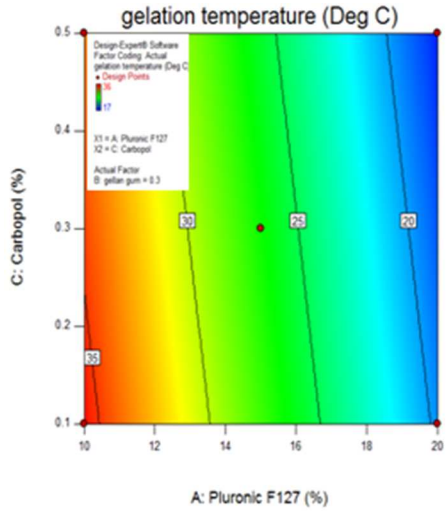

Fig. 8: Contour plot of the effect of pluronic *F-127* (A) and carbopol (C) on gelation temperature

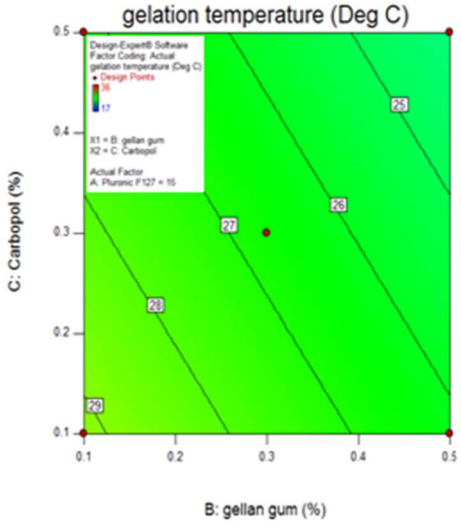

Fig. 9: Contour plot of the effect of gellan-gum (B) and carbopol (C) on gelation temperature

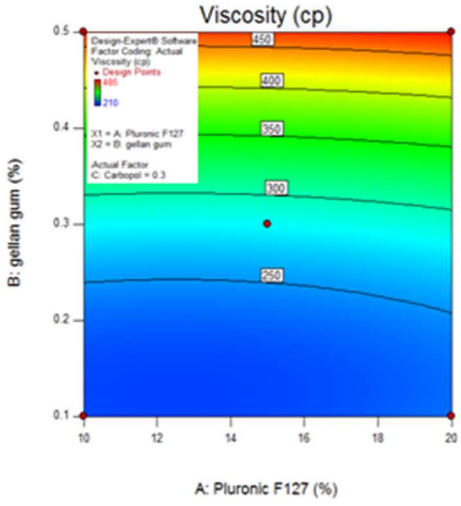

Fig. 10: Contour plot of the effect of pluronic *F-127* (A) and gellan-gum (B) on the viscosity

Fig. 10 shows the effect of factor A and B on response y3 while keeping factor C at a constant level. The positive relation of factor B with response y3 (viscosity) has observed whereas, factor A has not shown the significant effect on response y3. Fig. 11 shows the effect of factor A and C on response y3 by keeping factor B at a constant level. Factor C showed the positive effect on response y3 (viscosity), and comparatively the factor A has a very low effect on response y3. Moreover, fig. 12 shows the effect of factor B and C on response y3 while keeping factor A at a constant level. Factor B and C indicates the positive relation between response y3.

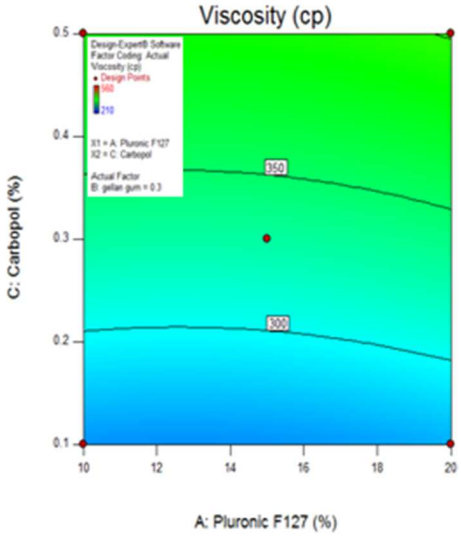

Fig. 11: Contour plot of the effect of pluronic *F-127* (A) and carbopol (C) on the viscosity

Gelation temperature is further important to increase the therapeutic efficacy of the product by increasing the moisture uptake and prevent the washing of dose [21]. The gelation temperature decreased with increase in concentration and molecular weight of gellan-gum. Increase in concentration of gellan-gum further reduced the overall concentration of pluronic which was the main factor for controlling the gelation temperature whereas, increased in molecular weight of pluronic insignificantly increases the gelation temperature.

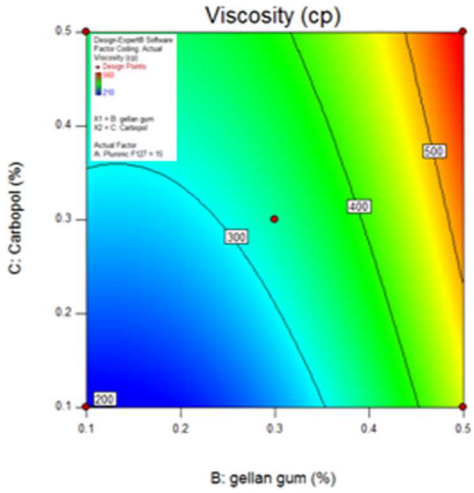

Fig. 12: Contour plot of the effect of gellan-gum (B) and carbopol (C) on the viscosity

Optimization of formulation using a graphical optimization method

Optimization of the formulation was performed to determine the levels of factors A, B, C where yield response of y1 was 2.8 to 3.1 hr, y2 was 30 to 36 and y3 was 150 to 350 m. Pa. S. This model predicted y1, y2 and y3 in required range at A, B, and C values of 11.50 (g), 0.32 (g), 0.3 (g) respectively for a batch size of 100 g. On the basis of these values, three different batches of the in-situ gel were prepared and found that the obtained values were the very close agreement to the predicted values, which establishes the reliability of the optimization process. In fig. 13 the overlay plot has shown the optimised formulation as suggested by design expert software for desired range response.

Finally, the optimised concentration of variable factors was found to be, plurionic (11.50% w/v), gellan-gum (0.32% w/v), carbopol (0.3% w/v) and the final formulation is presented in table 8.

Table 8: Final optimised formulation details

S. No.	Ingredients	Quantity (g)
1	Moxifloxacin hydrochloride	0.3
2	Pluronic *F-127*	11.50
3	Gellan-gum	0.32
4	Carbopol	0.3
5	Benzalkonium Chloride	0.006
6	Distilled Water	Quantity sufficient for 100 g

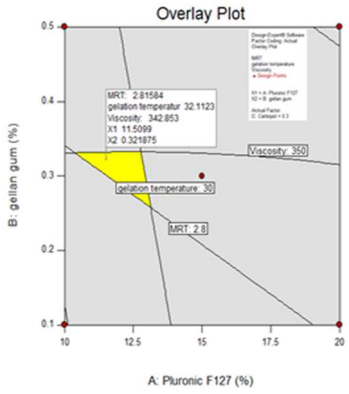

Fig. 13: Overlay plot for optimized parameter of *in-situ* hydrogel

Accelerated stability study

Accelerated stability studies of the optimized formulation were analysed periodically, but no significant change was observed in the visual appearance, pH, gelling capacity, drug content and *in vitro* drug release which confirm the stability of the optimized formulation for the long duration.

Antimicrobial activity

Antibacterial activity of optimized formulation was compared against the marketed formulation. Table 9 showed the zone of inhibition (ZOI) obtained by the prepared formulation of moxifloxacin hydrochloride as compared to the marketed formulation. The higher ZOI obtained by synthesized formulation indicate the potency of formulation that can relate the higher viscosity of formulation which results in slow and prolong the release of the formulation.

Table 9: Anti-bacterial potency of the synthesized and marketed formulation

Formulation	Zone of inhibition (Diameter in mm)	
	Pseudomonas aueroginosa	***Staphylococcus aureus***
Selected Formulation	31.4±0.8	31.1±0.7
Marketed Formulation	31.3±1.4	30.2±1.2

*All values were represented as mean±SD (n=3).

Ocular irritancy assay

The equivalent dose of optimized formulation against marketed formulation has shown no ocular irritancy on rabbit eyes and for the duration of consecutive 14 d. No watering of eyes, mucosal discharge and swelling were observed during the defined period of time which showed the biocompatibility and no irritancy of optimized formulation in the biological system.

CONCLUSION

Optimization of the in-situ formulation using pluronic F-127, carbopol, gellan-gum was a complex process since it involves a large number of variables, which affect the characteristics of the final formulation. The response surface method was successfully applied for formulation optimization. The concentration of pluronic F-127, carbopol, and gellan-gum, plays a significant role in influencing MRT, gelation temperature and viscosity of the desired product. The graphical optimization helps in identifying the sweet spot, having all the desired physiochemical properties. The study also helps in providing crucial data for scale-up study or large-scale production. Gelation temperature was found to be more dependent on pluronic concentration and the MRT and viscosity of the product were found to be more dependable in the concentration of carbopol and gellan-gum. The supportive study including accelerated stability study, antimicrobial assessment, and ocular irritancy assay proved the long durability with high potency and no irritancy of the optimized formulation.

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ABBREVIATION

Mean release time; MRT, Microbial Type Culture Collection; MTCC, Analysis of variance; ANOVA, Predicted Residual Error Sum of Squares; PRESS, the degree of freedom; df.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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