Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1254-1264 | ISSN:2251-6727

Development and Optimization of Moxifloxacin-Loaded in-Situ Gel Using Box-Behnken Design for Ophthalmic Drug Delivery

Anjali Kumarawat[1]*, Nadeem A. Farooqui[2], Darshan Jamindar[3], Nimita Manocha[4]

Department of Pharmaceutics, Indore Institute of Pharmacy, Pithampur Road, Opp. IIM, Rau, Indore-453331(M.P.) India.

***Corresponding author**- Anjali Kumarawat, Department of Pharmaceutics, Indore Institute of Pharmacy, Pithampur Road, Opp. IIM, Rau, Indore-453331(M.P.) India.

(Received: 04 February 2024 Revised: 11 March 2024 Accepted: 08 April 2024)

KEYWORDS

In-situ gel, ophthalmic drug delivery, novel approach, Box-Behnken design.

ABSTRACT:

Objective: This research aims to develop, optimize and characterize the sustained release *in situ* ocular gel containing moxifloxacin hydrochloride. **Material and Method:** Box-Behnken design was applied to optimize the formulation. The independent variables were (X_1) concentration of sodium alginate (%w/v), (X_2) concentration of carbopol 934 (%w/v) and (X_3) concentration of HPMC K100M (%w/v) while the (Y_1) drug release (%) was the dependent variable.

Evaluation: Further the optimized formulation was evaluated for appearance, pH, viscosity, gelling capacity, drug content estimation, sterility testing and spreadability of formulated gel.

Result: The optimized formula of moxifloxacin *in situ* gel containing sodium alginate (1.5 %w/v), Carbopol 934 (1%w/v) and HPMC K100M (0.625%w/v) was found to be clear, pH was found to be in range (6.8-7.4) and showed maximum drug release of 96.45% upto 8 hours. These results concluded that *in situ* gels can be used as an alternative of traditional eye drops.

Introduction

Ocular drug delivery is a challenging task for the pharmaceutical scientists because of the protective mechanism of eyes such as rapid precorneal elimination of drug, tear turnover and poor drug bioavailability.[1] Conventional drug delivery system such as eye drops shows poor ocular bioavailability (<5%). To overcome these factors, the designing of a novel approach for simple, safe, and effective drug delivery has been investigated.[2] Over the past decades several novel approaches have been developed among which *in-situ* gels has attained a great attention which were investigated by researchers to enhance retention time, bioavailability and therapeutic efficacy of the topically administered drugs.[3]

When the polymer chain is cross-linked, either chemically (chemical cross-linking) or non-covalently (physical cross-linking), the polymeric solution transforms into the gel. An additional mechanism known as physiologic stimuli are also responsible for the formulation of *in-situ* gel, these stimuli include those that are triggered by temperature and pH. [4] Numerous natural and synthetic polymers such as alginic acid, gellan gum, xanthum gum, xyloglucan, pectin, chitosan, poly (DL lactic acid), poly (DL-lactide-co-glycolide), and poly-caprolactone are employed in the formulation and development of *in-situ* gel drug delivery systems.[5]

The formulation adopted is a sodium alginate solution containing carbopol 934 and HPMC K 100 M (viscosity enhancer) which complexes with the Ca^{2+} ions present the lachrymal fluid. Free calcium ions interact with

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1254-1264 | ISSN:2251-6727

sodium alginate, leading to cross-linking of polymer chains and forming a matrix structure. Gelation involves the formation of double helical junction zones and reassembly of helical segments, creating a three-dimensional network via cation complexation and hydrogen bonding with water.

Moxifloxacin acts as an antibacterial agent by targeting and inhibiting the functions of DNA gyrase (also known as topoisomerase II) and topoisomerase IV. These enzymes are crucial for bacterial DNA processes, including replication, translation, repair, and recombination. By binding to these enzymes, Moxifloxacin disrupts the survival mechanisms of bacteria, making it an effective concentration-dependent, bactericidal anti-infective medication.[6]

Materials and Methods

Materials

Moxifloxacin Hydrochloride was obtained from MSN Pharmachem Private Limited, Hyderabad as a gift sample. Sodium alginate was procured from Sisco Research Laboratory Pvt. Ltd. Maharashtra. Carbopol 934 was purchased from Loba Chemie Pvt Ltd, Mumbai. HPMC K100M was procured from Chemdyes Corporation, Rajkot Gujarat. Other ingredients and reagents were used of analytical grade.

Methods

Drug-Polymer Compatibility Studies

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra for moxifloxacin hydrochloride in its pure form alongside a mixture containing moxifloxacin hydrochloride, carbopol 934, and HPMC K100M were recorded with the use of an FTIR Spectrophotometer. To evaluate the physicochemical interactions between the drug and its excipients, analysis through FTIR was conducted utilizing the Potassium bromide disk technique.[7]

Method of Preparation of *In-situ Gel*

The composition of *in-situ* gel containing moxifloxacin hydrochloride was prepared by employing Box-Behnken design presented in Table 2. To prepare these formulations, Sodium alginate combined with Carbopol® 934 and HPMC K100M was utilized using the dispersion method. Initially, 75 ml of distilled water was preheated to 70°C to dissolve sodium alginate, followed by the addition of HPMC K100M, and Carbopol® 934 into the solution. This mixture was left at room temperature overnight to allow for polymer hydration. Separately, moxifloxacin hydrochloride (0.5%w/v) was dissolved in 25 mL of distilled water and then added to the polymeric solution. The mixture was stirred until a uniform solution was achieved. The final product was then filled into sterile amber-colored bottles and sterilized in an autoclave at 121°C for 15 minutes. Subsequently, the prepared formulations were stored in a refrigerator at 4°C until further use.[6],[8]

Formula Optimization using Design Expert Software

Computer-aided modeling-

Box–Behnken statistical design with 3-factors, 3-levels, and 15 runs was implemented for the optimization study using Design-Expert software (version 13). Concentration of sodium alginate (%w/v) (X1), concentration of carbopol 934 (%w/v) (X2) and Concentration of HPMC K100M (%w/v) (X3) were selected as independent variables and they were set at low, medium and high levels. Table no.1 summarizes the coded values of different variables. In accordance with the design, 15 *in-situ* gel formulations were prepared and characterized for *in-vitro* % drug release (Y1) which was chosen as response parameter. This design explains the main effects and interaction effects of the independent variables on the formulation characteristics. The objective function for the present study was to enhance the retention time of the formulation and sustained drug release of the product.

$$Y1 = b_0 + A + B + C \quad \text{(Equation 1)}$$

Where, b_0 is arithmetic mean response of 15 runs, A, B, and C are the independent variables.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1254-1264 | ISSN:2251-6727

Table1. Levels and Constrains of Independent and Dependent Variables

Levels and Constrains of Independent and Dependent Variables				
Independent Variables	Levels			Constrains
	Low (-1)	Medium (0)	High (+1)	
A: Concentration of Sodium Alginate	0.5	1	1.5	
B: Concentration of Carbopol 934	0.25	0.625	1	
C: Concentration of HPMC K100M	0.25	0.625	1	
Dependent Variables	Drug Release upto 8 hours (%)			>95%

Data analysis and validation of model

Box–Behnken design was particularly selected, because it needs fewer runs than a central composite design, in cases of three or four variables. Analysis of variance (ANOVA) was used to establish the statistical validation of the polynomial equations generated by Design Expert software. All the responses observed were simultaneously fitted to linear (first order), and quadratic models. Various feasibilities were conducted over the experimental domain to find the compositions of the optimized *in-situ* gel formulation. 2D and 3D response surface plots (fig.4 and fig.5) were generated by the Design Expert software. The resultant experimental values of the responses were quantitatively compared to that of the predicted values.[9]

Characterization of Moxifloxacin loaded *in-situ* gel

1. Physical Appearance

The formulated *in-situ* gel solutions were tested for clarity by visual appearance against black and white background in bright light. The pH of the prepared in-situ gel was measured by digital pH meter (Labtech DPH-115 PM, Chennai).[10]

2. Gelling Capacity

The ability of the formulation to form a gel was evaluated by adding several drops of it into a vial with 2 ml of simulated tear fluid, freshly prepared and with a pH of 7.4. Observation was made visually to determine the duration needed for gel formation.[11]

3. Rheological Studies

The viscosity estimation was carried out using Brookfield viscometer (DV-E, USA model). The *in- situ* gel formulations were placed in a beaker. The samples were analyzed at 27°C. Spindle number 61 was dropped perpendicularly into the solution in a beaker. The angular velocity of the spindle was increased from 10 rpm to 30 rpm and the rheological behaviour of the formulation was noted at the end of 30 seconds.[12]

4. Spreadability

To determine the spread ability of in-situ gel, transfer 1g of gel to the centre of a glass plate (10cm×10cm) at 32 ± 0.5ºC and cover this plate by another glass plate of same size. Apply some weight (125g) on the upper slide to evenly spread the gel. After one minute remove the weight and measure the diameter of the gel spread area (cm). Perform this determination test in triplicates. The mathematical formula expressed as:

$$S = M \times L / T$$

Where S is spreadability, M is weight tied to the upper glass slide, L is length of the glass slide and T is time taken to spread the gel.[13]

5. Drug Content Estimation

The % drug content estimation was carried out by diluting prepared formulation equivalent to 50mg in 50 ml of distilled water and analyzed after further dilution

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1254-1264 | ISSN:2251-6727

using UV-visible spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Japan) at 289 nm. The experiment was performed in triplicates.[14]

6. *In-vitro* Dissolution Studies

The in vitro release of moxifloxacin hydrochloride from the formulated preparation was investigated using a Franz diffusion cell apparatus, comprising two compartments: a donor compartment and an acceptor compartment. The acceptor compartment was filled with freshly prepared simulated tear fluid (pH 7.4), maintained at a temperature of 37°C±1°C with continuous stirring using a magnetic stirrer. Subsequently, the donor compartment, containing a cellophane membrane soaked overnight, was securely attached to the acceptor compartment. A 2 ml volume of the prepared solution was then added to the donor compartment. At predetermined time intervals, 1 ml aliquots were withdrawn from the acceptor compartment and replaced with fresh simulated tear fluid. All samples were collected in triplicate. The amount of drug released was quantified using a UV-visible spectrophotometer at 289 nm, with simulated tear fluid at pH 7.4 serving as the blank control.[15]

7. Sterility Test

To ensure the sterility of ocular products, it is essential to conduct sterility testing. In this process, the chosen formulation is transferred into a sterile fluid thioglycolate medium under aseptic conditions. The mixture is then incubated for a minimum of 14 days at 35°C to monitor bacterial growth. Sterility is assessed by visually inspecting the clarity of the medium over the 14-day period.[16]

Result and Discussion

Drug-Polymer Compatibility Studies

Fourier Transform Infrared Spectroscopy (FTIR)

An infrared analysis was conducted to investigate potential interactions between the pure drug and additives. Figure 1 depicts the FTIR spectra of moxifloxacin hydrochloride along with physical mixture.

The spectra of pure moxifloxacin hydrochloride (MH) displayed distinct peaks, including C=O stretching vibrations at 1710 cm^{-1}, N-H stretching vibrations at 2928 cm^{-1}, and O-H stretching vibration at 3527 cm^{-1}. In FTIR spectra of physical mixture, primary absorption bands of moxifloxacin hydrochloride were evident, showing no notable alterations compared to pure drug spectrum. This observation indicates no apparent interaction between the drug and polymers.

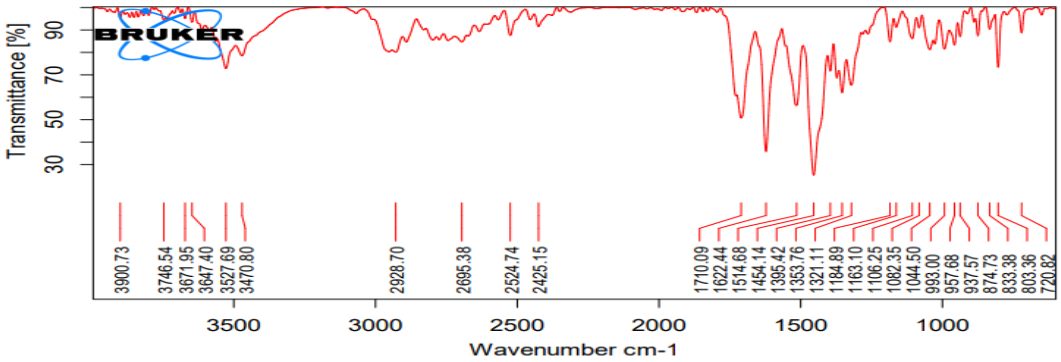

Fig.1 FTIR spectra of pure moxifloxacin hydrochloride (API)

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1254-1264 | ISSN:2251-6727

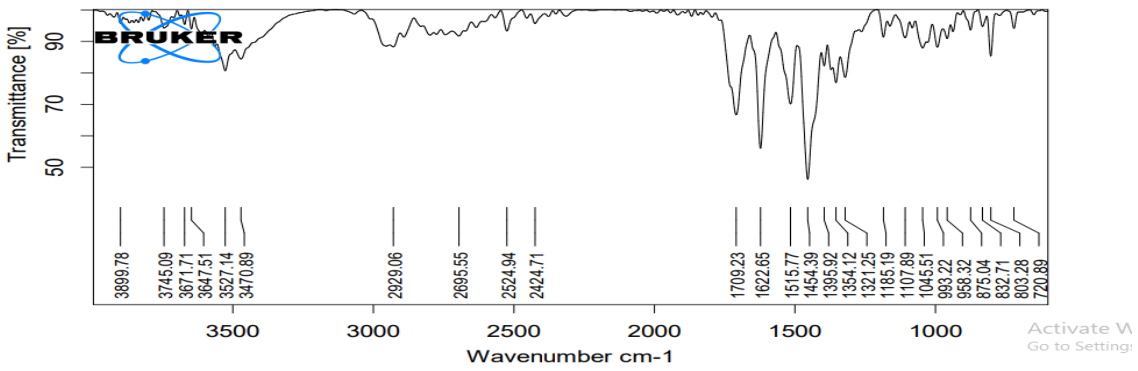

Fig.2 FTIR spectra of physical mixture of moxifloxacin hydrochloride and polymers

Formula Optimization using Design Expert Software

Box–Behnken statistical design with 3-factors, 3-levels, and 15 runs was employed for the optimization study using the Design Expert software (version 13). In accordance with the design, 15 *in-situ* gel formulations were prepared and characterized for *in-vitro* % drug release (Y1) which was chosen as response parameter. (Table 2)

Table 2. Experimental run by design expert software

		Factor 1	**Factor 2**	**Factor 3**	**Response 1**
Std	Run	A:Concentration of Sodium Alginate	B:Concentration of Carbopol 934	C:Concentration of HPMC K100M	Drug Release upto 8 hours
		%w/v	%w/v	%w/v	%
1	1	0.5	0.25	0.625	78.67
15	2	1	0.625	0.625	85.94
9	3	1	0.25	0.25	82.74
13	4	1	0.625	0.625	87.01
12	5	1	1	1	89.54
3	6	0.5	1	0.625	80.02
4	**7**	**1.5**	**1**	**0.625**	**96.45**
11	8	1	0.25	1	85.26
14	9	1	0.625	0.625	85.71
5	10	0.5	0.625	0.25	77.63
10	11	1	1	0.25	88.35
2	12	1.5	0.25	0.625	93.54
7	13	0.5	0.625	1	80.92
6	14	1.5	0.625	0.25	94.32
8	15	1.5	0.625	1	95.02

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1254-1264 | ISSN:2251-6727

Data Analysis and Optimization

Response Y1: Drug Release upto 8 hours

The high value of the correlation coefficient indicates a good fit between the independent variables, and the dependent variable. The linear model was found to be significant as shown in (Table 3) and the P value is less than < 0.05. This result clearly demonstrates that this selected independent variable have a statistically significant influence on the drug release.[17]

Table 3. Analysis of Variance (ANOVA for Linear model)

Source	Sum of Squares	Degree of Freedom	Mean Square	F-value	p-value	
Model	514.34	3	171.45	193.47	< 0.0001	**significant**
A-Concentration of Sodium Alginate	481.90	1	481.90	543.79	< 0.0001	
B-Concentration of Carbopol 934	25.03	1	25.03	28.24	0.0002	
C-Concentration of HPMC K100M	7.41	1	7.41	8.36	0.0147	
Residual	9.75	11	0.8862			
Lack of Fit	8.79	9	0.9761	2.03	0.3737	**not significant**
Pure Error	0.9626	2	0.4813			
Cor Total	524.08	14				

The Model F-value of 193.47 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, and C are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The Lack of Fit F-value of 2.03 implies the Lack of Fit is not significant relative to the pure error. There is a 37.37% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Table 4. Showing Fit Statistics of linear model (ANOVA)

Fit Statistics of linear model (ANOVA)			
Std. Dev.	0.9414	**R^2**	0.9814
Mean	86.74	**Adjusted R^2**	0.9763
C.V. %	1.09	**Predicted R^2**	0.9635
		Adeq Precision	39.2084

The Predicted R^2 of 0.9635 is in reasonable agreement with the Adjusted R^2 of 0.9763; i.e. the difference is less than 0.2.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1254-1264 | ISSN:2251-6727

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 39.208 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Actual Factors

Drug release (Y1) = 66.66675 + 15.52250*A + 4.71667*B + 2.56667*C

The design expert software suggested that 1.49% of sodium alginate, 0.85% of carbopol 934 and 0.983% of HPMC K100 shows optimum drug release upto 8 hours. All response graphs showing desirability and drug release at different independent variables are shown in figure 3. Contour plot and three dimensional response surface plot of optimized formulation as shown in figure 4 and 5 respectively shows the interaction effects of the factors on response. When the experimental data of response (Y1) of 15 runs was fitted into different models of Box-Behnken design, linear model was found to be best fitted model.

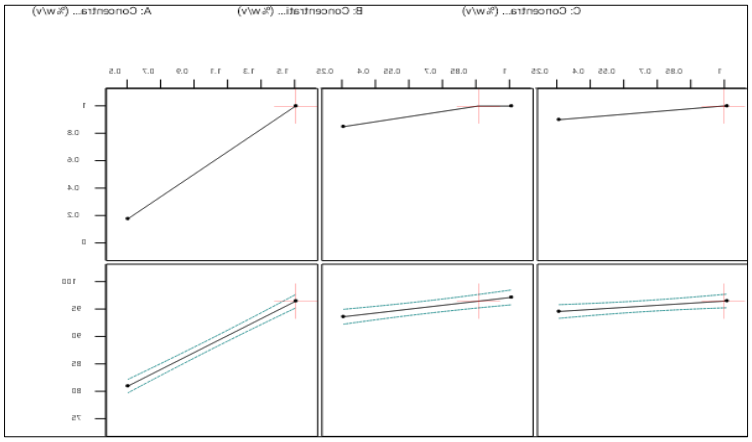

Fig.3 All response graphs showing desirability and drug release at different independent variables

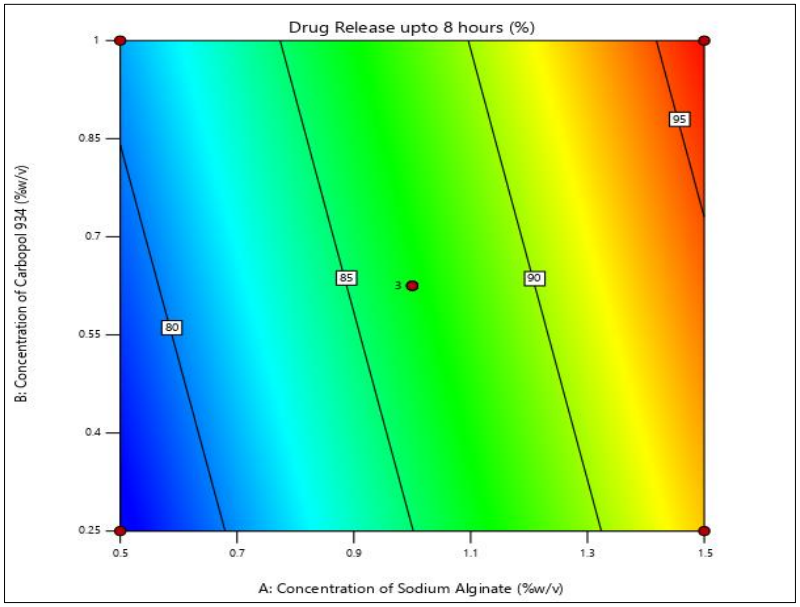

Fig.4 The Contour plot for Y_1 (% Drug release upto 8 hours)

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1254-1264 | ISSN:2251-6727

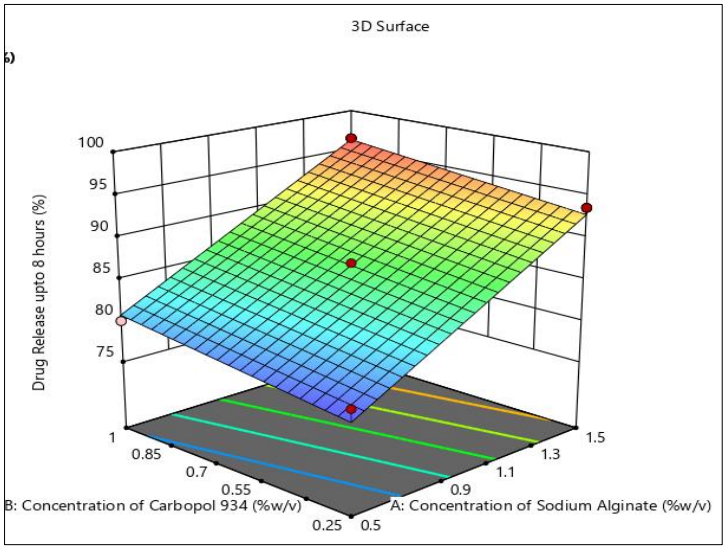

Fig.5 The 3D response surface plot showing effect of independent variable on Y_1 (% Drug release upto 8 hours)

Evaluation of *in-situ* gel

Physical Parameter

The optimized prepared formulation of *in-situ* gel (F7) was found to be transparent and clear. The pH of the formulation was found to be in range between 6.8 and 7.4 when noted in triplicates (n=3).

Gelling capacity

Stiff gel of optimized formula F7 is formed immediately and remained for extended period of time (more than 8 hours).

Rheological studies

Viscosity of the ophthalmic *in-situ gel* is an important parameter to determine the residence period of drug into the eyes. The viscosity of polymeric solution was found to be 580cp±0.84 before conversion into gel and the viscosity of *in-situ gel* was found to be 1450cp±0.57 after becoming the gel which comes under the preferred range.

Spreadability

The spreadability of optimized *in situ* gel was found to be 13.95±0.43 g.cm/sec which was in the acceptable range. Spreadability is inversely proportional to viscosity. Increase in concentration of any polymer can decrease the spreadability of the formulation.

Drug content

The drug content of optimized *in situ* gel was analyzed in triplicates by UV-Visible spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Japan) at 289nm and the drug content was found to be 97.47±0.2 %.

***In-vitro* dissolution studies**

This study was performed by Franz diffusion cell for 8 hours. By this study it was concluded that an increase in the concentration of polymers shows the release retarding property of the polymer.

Table 5. *In-vitro* drug release studies of optimized batch

***In-vitro* drug release studies of optimized batch**				
S.No.	**Time (hours)**	**% Drug Release**		
		F7 (Optimized)	**F14**	**F15**
1	**0**	**0**	**0**	**0**
2	1	3.62±0.02	3.16±0.15	3.46±0.05

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1254-1264 | ISSN:2251-6727

3	2	20.52±0.32	16.41±0.81	14.6±0.12
4	3	34.78±0.45	33.28±0.32	31.77±0.67
5	4	48.22±0.27	42.92±0.25	40.51±0.82
6	5	67.74±0.32	58.89±0.41	56.78±0.16
7	6	78.09±0.12	70.93±0.23	68.52±0.90
8	7	83.59±0.83	82.08±0.68	80.57±0.24
9	8	96.45±0.91	95.03±0.24	94.31±0.54

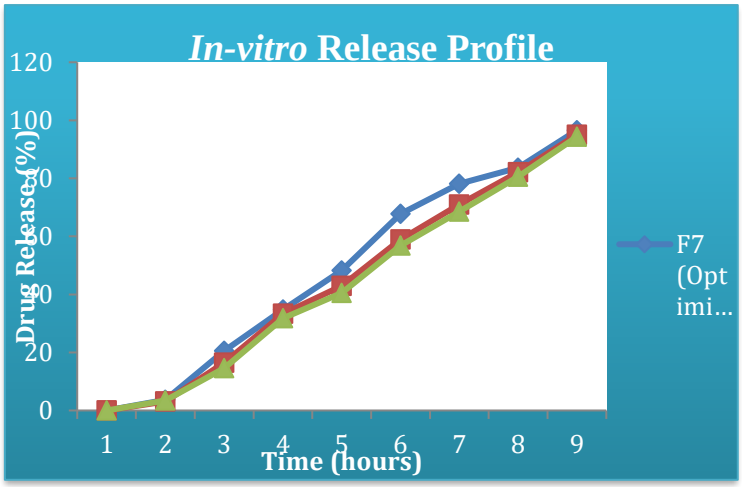

Fig.6 *In vitro* release profile of optimized moxifloxacin hydrochloride *in-situ* gel (F7, F14 and F15)

Data Analysis via Drug release Kinetics Studies

The formulation of moxifloxacin *in-situ* gel was subjected to drug release studies using Franz Diffusion Cell apparatus containing simulated tear fluid (pH 7.4) and cellophane membrane. The fitting of drug dissolution profile against kinetics models were analyzed using Microsoft Excel. It was observed that the optimized batch F7 followed First Order Release Kinetics as the plot showed linearity.

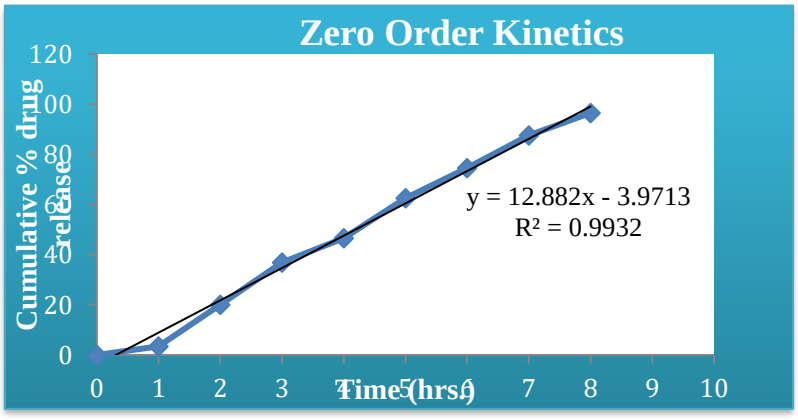

Fig.7 Zero Order Release Kinetics of Optimized formulation (F7)

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1254-1264 | ISSN:2251-6727

Sterility Test

To ensure the sterility of ocular products, it is essential to conduct sterility testing. In this process, the chosen formulation F7 was transferred into a sterile fluid thioglycolate medium under aseptic conditions. The mixture was then incubated for a minimum of 14 days at 35°C to monitor bacterial growth. Sterility was assessed by visually inspecting the clarity of the medium over the 14-day period.

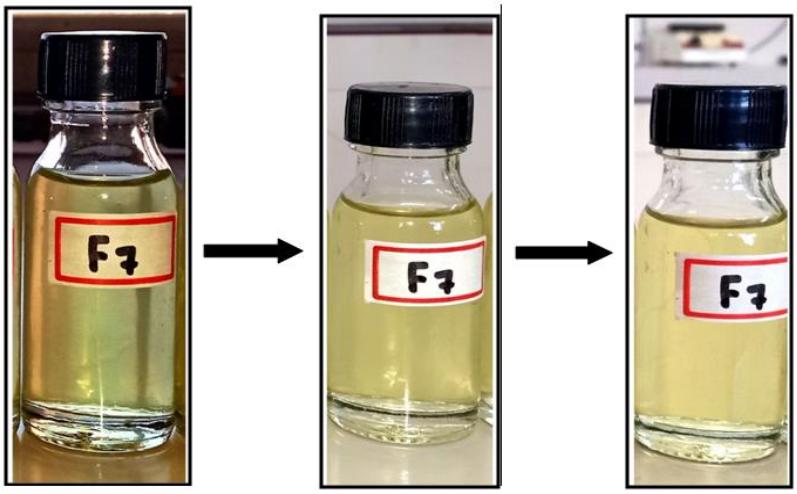

Fig.7 Sterility testing of optimized formulation F7 from day 1 to day 14.

Conclusion

The present study demonstrate that the optimized *in-situ gel* of moxifloxacin hydrochloride was prepared successfully using sodium alginate, carbopol 934 and HPMC K100M for ophthalmic drug delivery that showed sustained drug release for more than 8 hours and enhanced retention time. Certainly Commercialization of a dosage form requires both preclinical and clinical studies. To optimize an in situ ophthalmic gel, a Box-Behnken design was employed. The optimization analysis determined that a batch comprising 1.5%w/v sodium alginate, 1%w/v carbopol 934 and 0.625% HPMC K100M exhibited the longest residence time and improved efficacy. On the basis of all evaluation parameters it was concluded that ophthalmic *in-situ* gel shows potential as a promising delivery system for ophthalmic drugs, offering enhanced bioavailability.

Acknowledgement

The authors are grateful to MSN Pharma Chem Private Limited, Hyderabad for providing moxifloxacin as gift sample. I wish to express my sincere gratitude to the Indore Institute of Pharmacy, Indore for their invaluable support, and guidance, all of which played a crucial role in the publication of my research article. Finally, I would like to extend my sincere appreciation to the journal for accepting and publishing our work.

Conflict Of Interest

The authors declare no conflict of interest.

Reference

1. Gaudana, R.; Jwala, J.; Boddu, S. H. S.; Mitra, A. K. Recent Perspectives in Ocular Drug Delivery. *Pharm. Res.* **2009**, *26* (5), 1197.
2. Araújo, J.; Gonzalez, E.; Egea, M. A.; Garcia, M. L.; Souto, E. B. Nanomedicines for Ocular NSAIDs: Safety on Drug Delivery. *Nanomedicine Nanotechnol. Biol. Med.* **2009**, *5* (4), 394–401.
3. Paul, S.; Majumdar, S.; Chakraborty, M. Revolutionizing Ocular Drug Delivery: Recent Advancements in in Situ Gel Technology. *Bull. Natl. Res. Cent.* **2023**, *47* (1), 154.
4. Vigani, B.; Rossi, S.; Sandri, G.; Bonferoni, M. C.; Caramella, C. M.; Ferrari, F. Recent Advances in the Development of In Situ Gelling Drug Delivery Systems for Non-Parenteral Administration Routes. *Pharmaceutics* **2020**, *12* (9), 859.
5. Sabale, A. S.; Kulkarni, A. D.; Sabale, A. S. Nasal In Situ Gel: Novel Approach for Nasal Drug

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1254-1264 | ISSN:2251-6727

Delivery. *J. Drug Deliv. Ther.* **2020**, *10* (2-s), 183–197.

6. Dholakia, M.; Dave, R.; Thakkar, V.; Rana, H.; Gohel, M.; Patel, N. Newer Ophthalmic In Situ Gel Of Moxifloxacin Hydrochloride: Optimization Using Box Behnken Statistical Design. *Int. J. Pharm. Pharm. Sci.* **2018**, *10* (12), 5.
7. Kalaria, V. J.; Saisivam, S.; Alshishani, A.; Aljariri Alhesan, J. S.; Chakraborty, S.; Rahamathulla, M. Design and Evaluation of *in Situ* Gel Eye Drops Containing Nanoparticles of Gemifloxacin Mesylate. *Drug Deliv.* **2023**, *30* (1), 2185180.
8. Mandal, S.; Prabhushankar, G.; Thimmasetty, M.; Geetha, M. Formulation and Evaluation of an in-situ Gel-Forming Ophthalmic Formulation of Moxifloxacin Hydrochloride. *Int. J. Pharm. Investig.* **2012**, *2* (2), 78.
9. Baig, M. S.; Ahad, A.; Aslam, M.; Imam, S. S.; Aqil, Mohd.; Ali, A. Application of Box–Behnken Design for Preparation of Levofloxacin-Loaded Stearic Acid Solid Lipid Nanoparticles for Ocular Delivery: Optimization, in Vitro Release, Ocular Tolerance, and Antibacterial Activity. *Int. J. Biol. Macromol.* **2016**, *85*, 258–270.
10. Jain, D.; Kumar, V.; Singh, S.; Mullertz, A.; Shalom, D. B. Newer Trends in In Situ Gelling Systems for Controlled Ocular Drug Delivery. *J. Anal. Pharm. Res.* **2016**, *2* (3), 98–111.
11. Kaur, H.; Loyee, S. L.; Garg, R. Formulation and Evaluation of In-Situ Ocular Gel of Gatifloxacin. *Int. J. Pharma Res. Health Sci.* **2016**, *4* (5), 1365–1370.
12. Kondepati, H. V.; Kulyadi, G. P.; Tippavajhala, V. K. A Review on *In Situ* Gel Forming Ophthalmic Drug Delivery Systems. *Res. J. Pharm. Technol.* **2018**, *11* (1), 380.
13. Chaudhary, B.; Verma, S. Preparation and Evaluation of Novel *In Situ* Gels Containing Acyclovir for the Treatment of Oral Herpes Simplex Virus Infections. *Sci. World J.* **2014**, *2014*, 1–7.
14. Kolawole, O. M.; Cook, M. T. In Situ Gelling Drug Delivery Systems for Topical Drug Delivery. *Eur. J. Pharm. Biopharm.* **2023**, *184*, 36–49.
15. Makwana, S. B.; Patel, V. A.; Parmar, S. J. Development and Characterization of In-Situ Gel for Ophthalmic Formulation Containing Ciprofloxacin Hydrochloride. *Results Pharma Sci.* **2016**, *6*, 1-6.
16. Sheshala, R.; Ming, N. J.; Kok, Y. Y.; Raj Singh, T. R.; Dua, K. Formulation and Characterization of pH Induced in Situ Gels Containing Sulfacetamide Sodium for Ocular Drug Delivery: A Combination of Carbopol®/ HPMC Polymer. *Indian J. Pharm. Educ. Res.* **2019**, *53* (4), 654–662.
17. Fukuda, I. M.; Pinto, C. F. F.; Moreira, C. D. S.; Saviano, A. M.; Lourenço, F. R. Design of Experiments (DoE) Applied to Pharmaceutical and Analytical Quality by Design (QbD). *Braz. J. Pharm. Sci.* **2018**, *54*.

Figure Legends

Fig.1 FTIR spectra of pure moxifloxacin hydrochloride (API)

Fig.2 FTIR spectra of physical mixture of moxifloxacin hydrochloride and polymers

Fig.3 All response graphs showing desirability and Drug release at different independent variables

Fig.4 The response Contour plot for Y_1 (% Drug release upto 8 hours)

Fig.5 The response Contour plot for Y_1 (% Drug release upto 8 hours)

Fig.6 *In vitro* release profile of optimized moxifloxacin hydrochloride *in-situ* gel (F7, F14 and F15)

Fig.7 Zero Order Release Kinetics of Optimized formulation (F7)

Fig.8 Sterility testing of optimized formulation F7 from day 1 to day 14.

Table Legends

Table1. Levels and Constrains of Independent and Dependent Variables

Table 2. Experimental run by design expert software

Table 3. Analysis of Variance (ANOVA for Linear model)

Table 4. Showing Fit Statistics of linear model (ANOVA)

Table 5. *In-vitro* drug release studies of optimized batch