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Research Article

DESIGN, DEVELOPMENT, AND EVALUATION OF AN ION-ACTIVATED OPHTHALMIC *IN SITU* GEL OF MOXIFLOXACIN HYDROCHLORIDE

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ABSTRACT

Objective: The objective of this study was to develop an *in situ* ophthalmic gel of an anti-infective drug, moxifloxacin (MOX) hydrochloride (HCL), for sustained ocular delivery for the treatment of bacterial infections of the eye.

Method: In the present work the *in situ* gelling systems were prepared by ion exchange method with the help of various concentrations of gelling agent gelrite (0.08 g, 0.1 g and 0.12 g) and sodium alginate (0.6 g, 0.8 g and 1 g) as viscosity enhancer were added in the formulation; 9 formulations were prepared according to 3^2 factorial designs and evaluated. The responses were analyzed for the analysis of variance using Design-Expert version 10 software. Statistical models were generated for each response parameter.

Results: Optimized formulation batch F7 (0.12% gelrite and 0.6% sodium alginate) was liquid before addition of simulated tear fluid (STF) and underwent rapid gelation on addition of STF and had given 84.05% cumulative drug release; the formulation was found to be clear, having good *in situ* gelling capacity, good antibacterial efficacy, having drug content 99.75%; optimized formulation was sterile and showed sustained drug release over 8 h period as compared to marketed eye drop.

Conclusions: From the above results, we can concluded that 3^2 full factorial design and statistical models can be successfully used to optimize the formulations, and it was concluded that the trial batch F7 (0.12% gelrite and 0.6% sodium alginate) is the best formula (percentage cumulative drug release over 84.05%) and it is possible to formulate *in situ* ophthalmic gels of MOX HCL using gelrite in combination with sodium alginate for the treatment of various bacterial infections of the eyes.

Keywords: Moxifloxacin hydrochloride, Gelrite, Sodium alginate, *In situ* gel, *In vitro* diffusion, Rheological studies.

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INTRODUCTION

Ophthalmic drug delivery is the most challenging and interesting area for upcoming pharmacists and formulation chemist due to unique anatomy and physiology of the eye. Eye drops that are conventional ophthalmic delivery systems often result in poor bioavailability and therapeutic response because high tear fluid turnover and dynamics cause rapid precorneal elimination of the drug. A high frequency of eye drop instillation is the main cause of patient non-compliance. Addition of excess drug in the formulation is an attempt to overcome bioavailability problem which is potentially dangerous if the drug solution drained from the eye is systemically absorbed from the nasolacrimal duct. Various other ophthalmic vehicles such as ointments, suspension, inserts, and aqueous gels have been developed to enhance the ophthalmic bioavailability. This ocular drug delivery system, however, has not been used frequently due to some disadvantages such as blurred vision from ointments or low patient compliance from inserts.

Above-mentioned problems can be overcome by the use of *in situ* gelling systems, a liquid dosage form suitable to be administered by instillation into the eye, which on exposure to physiological conditions, changes to the gel phase thus increase the precorneal residence time of the delivery system and enhance the ocular bioavailability. It comprises the ease of eye drop instillation and patient compliance as well as sustained release property that is described to intensify ocular bioavailability. The concept of *in situ* forming gels came into existence in the early 80s.

Depending on the method employed to cause sol to gel phase transition on the ocular surface, the following three types of systems have been recognized:

- pH-triggered - the polymers used in this system are pseudo-latexes - carbomer (carbopol), cellulose acetate phthalate latex.
- Temperature dependent - poloxamers (pluronic and tetronics), cellulose derivatives (methylcellulose and hydroxypropyl-methylcellulose), xyloglucan.
- Ion-activated induced – alginates and gelrite (gellan gum) [1].

Moxifloxacin (MOX) hydrochloride (HCL) is a fourth-generation fluoroquinolone broad-spectrum antibacterial, Biopharmaceutical Classification System Class-I drug, having low ocular bioavailability and therapeutic response due to high tear fluid turn over and rapid precorneal elimination of ocular dosage form. Hence, the rationale is to increase the bioavailability and patient compliance of MOX by formulating ophthalmic *in situ* gel which gives better residence time using a combination of polymers (as a release retardant) and make it more effective.

The aim of present work is to design, develop, and evaluate *in situ* ophthalmic gel of an anti-infective drug (MOX HCL 0.5% w/v) for sustained ocular delivery using a combination of gelrite as gelling agent and sodium alginate as viscosifying agent which is used for the treatment of various infective diseases of the eye, to get better patient compliance by increasing residence time and bioavailability.

The formulation would be useful to treat external infections of the eye such as acute and subacute conjunctivitis, bacterial keratitis, bacterial endophthalmitis, and keratoconjunctivitis.

MATERIALS AND METHODS

Materials

MOX HCL was purchased from Yarrow Chem Products Pvt., Ltd., Mumbai, Gelrite was provided by Yarrow Chem Products Pvt., Ltd., Mumbai.

Sodium alginate was purchased from Thomas Baker (Chemicals) Pvt. Ltd., Mumbai, and all other ingredients were of analytical grade.

Methods

Selection of drug and polymers

MOX is a fourth-generation fluoroquinolone with expanded activity against Gram-positive, Gram-negative bacteria as well as atypical pathogens. MOX is the hydrochloride salt of a fluoroquinolone antibacterial antibiotic. In common with quinolone antibiotics, the bactericidal action of MOX results from inhibition of the topoisomerase II (DNA gyrase) and topoisomerase IV required for bacterial DNA replication, transcription, repair, and recombination. Topoisomerase IV is the primary activity inhibited for many Gram-positive bacteria whereas DNA gyrase is the primary quinolone target in many Gram-negative microbes [2].

Determination of absorbance maxima of MOX by ultraviolet (UV) spectrophotometer

A solution of MOX containing the concentration 10 µg/ml was prepared in simulated tear fluid (STF) pH 7.4 and UV spectrum was taken using Shimadzu (UV-1800) double beam spectrophotometer. The solution was scanned in the range of 200–400 nm (Fig. 1).

Identification of MOX

Identification of MOX was carried out by Fourier-transform infrared (FTIR) spectrophotometer (Fig. 2).

Interaction studies [3]

The proper design and formulation of a dosage form require consideration of the physical, chemical, and biological characteristics of all drug substances and excipients to be used in the formulation of the product. The drug and excipients must be compatible with one another to produce a stable, efficacious, and safe product. The interaction study of prepared *in situ* gel formulations was carried out using IR spectroscopy following potassium bromide (KBr) dispersion method. The spectrum of a dried mixture of drug and KBr was then run followed by drug with excipients in the wavelength region between 4000 and 400 cm^{-1} (Fig. 4).

The drug-polymer compatibility was confirmed by differential scanning calorimetric (DSC). Thermal characterization of pure drug and polymer mixture was performed with a calorimeter, which was carried out by heating drug and the physical mixture of the drug with polymers

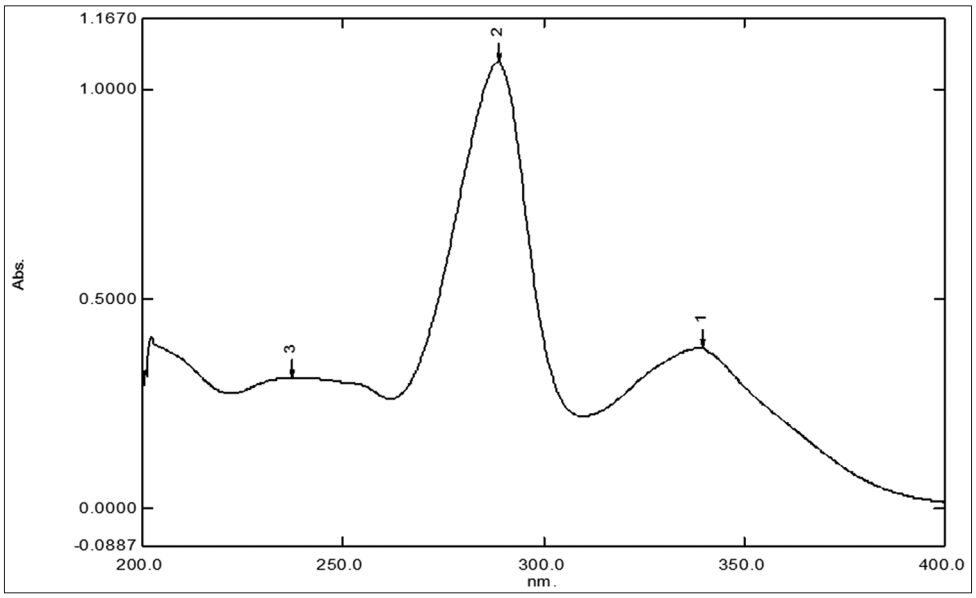

Fig. 1: Ultraviolet spectrum of moxifloxacin at 288.5

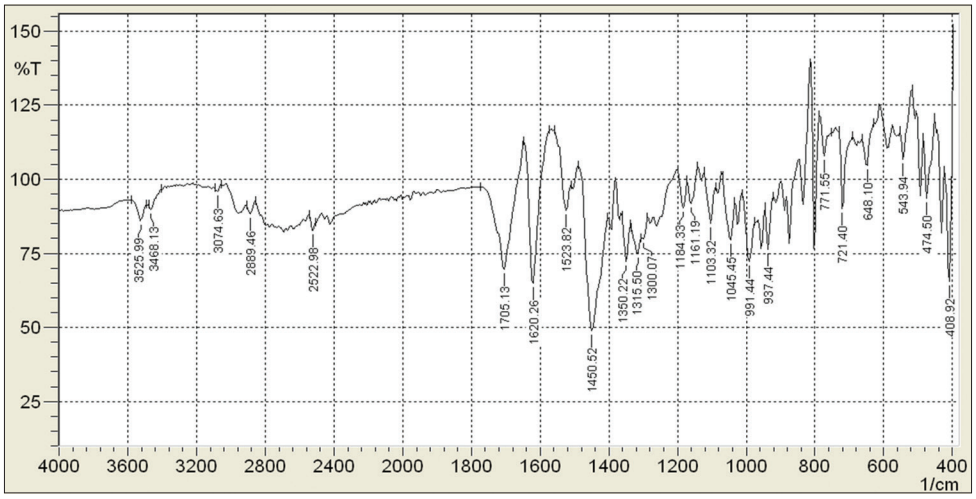

Fig. 2: Infrared spectra for pure moxifloxacin

separately from 20°C to 300°C at the heating rate of 10°C/min in a nitrogen environment (Fig. 5).

Preparation of *in situ* gelling system

Factorial design [4,5]

A 3^2 randomized full factorial design was used in the present study. In this design, 2 factors were evaluated; each at 3 level and experimental trials were performed for all 9 possible combinations. The concentration of gelrite (X1) and concentration of sodium alginate (X2) was chosen as an independent variable in 32 full factorial designs, while percent cumulative drug release was taken as dependent variable (Table 1a-c and Fig. 13).

The formulation layout for the factorial design batches (F1-F9) is shown in Tables 2 and 3.

Procedure

A 3^2 factorial design was used for formulation design, gellan gum (gelrite) and sodium alginate were chosen as an independent factor. Their effect on dependent factors such as drug release and viscosity was observed. An aqueous solution of a varying concentration of gelrite and sodium alginate was prepared and evaluated for gelling capacity and viscosity to identify the compositions suitable for as an *in situ* gelling system. Polymer solution was prepared by dissolving required quantity of sodium chloride in deionized water followed by dispersing gelrite and sodium alginate

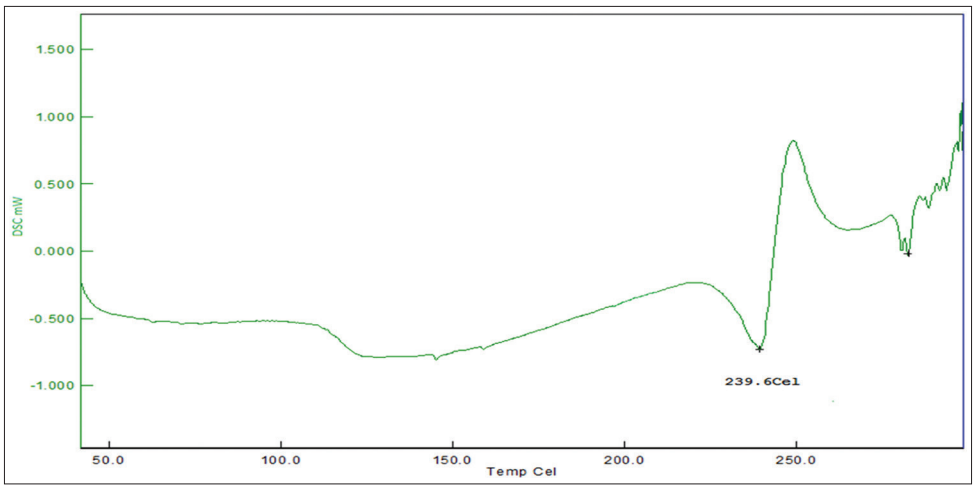

Fig. 3: Differential scanning calorimetric of hydrochloride

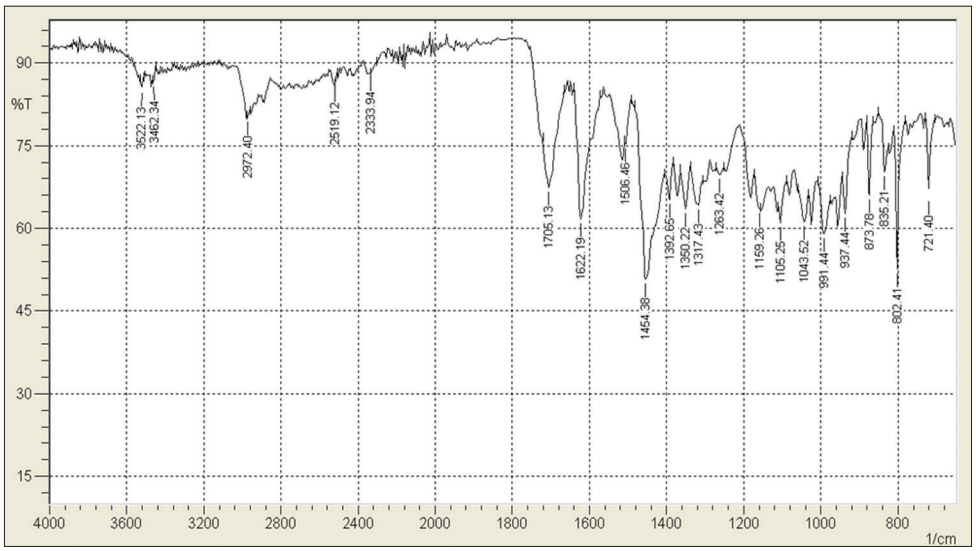

Fig. 4: Infrared spectra of a physical mixture of moxifloxacin, gelrite, and sodium alginate

Table 1: ANOVA for response surface linear model. (a): ANOVA table (partial sum of squares - type III)

Source	Sum of squares	df	Mean square	F value	p-value prob.>F	Significant
Model	417.56	2	208.78	107.32	<0.0001	
A-gelrite	346.56	1	346.56	178.14	<0.0001	
B-sodium alginate	71.00	1	71.00	36.50	0.0009	
Residual	11.67	6	1.95			
Cor total	429.23	8				

ANOVA: Analysis of variance

in above solution and heat up to 90°C for 20 min followed by cooling to room temperature, drug solution was prepared by dissolving MOX in mixture of propylene glycol and deionized water (8:100), drug solution was mixed with polymer solution using a magnetic stirrer under constant stirring until a uniform solution was obtained. The pH of the formulation was then set to 4.4 using 0.1 N HCL. The prepared *in situ* gels were filled in glass vials closed with rubber closures and sealed with aluminum caps and sterilized by autoclave at 121°C 15 psi for 20 mi [4,5].

Evaluation of prepared *in situ* gelling system

Interaction studies [6]

IR spectra were taken using FTIR spectrophotometer (Jasco 4100). The pellets of drug and KBr were prepared by compressing the powders (Ratio of drug to KBr 1:100) at 20 psi on KBr press, and the spectra were scanned in the wave number range of 4000–400 cm^{-1} FTIR study was carried on pure drug, physical mixture of drug and polymers, formulations to confirm the compatibility of drug with other excipients used in the preparation of *in situ* gels (Fig. 6).

Visual appearance and clarity [7]

Visual appearance and clarity were checked under fluorescent light against a white and black background for the presence of any particulate matter (Table 4).

Table 1b: Parameters of ANOVA (a)

Mean±SD	1.39±89.24
C.V. %	1.56
Press	24.6
−2 log likelihood	27.88
R-squared	0.9728
Adj. R-squared	0.9637
Pred. R-squared	0.9427
Adeq precision	27.419
BIC	34.47
AICc	38.68

SD: Standard deviation, ANOVA: Analysis of variance, Bayesian information criterion (BIC), and Akaike's information criterion

pH [8]

The pH of the prepared *in situ* gelling system after addition of all the ingredients was measured using pH meter (Table 4).

In vitro gelation [8]

The gelling capacity of formulations was evaluated to identify the formulations suitable for use as *in situ* gelling systems. Gelling capacity was determined by mixing the formulation with STF in the proportion 25:7 and examined visually.

The composition of STF was sodium chloride (0.68 g), sodium bicarbonate (0.22 g), calcium chloride dehydrate (0.008), potassium chloride (0.14 g), and double distilled water quantity sufficient up to100 g. Physiological pH (7.4±0.2) was adjusted by adding the required amount of 0.1 N HCl/NaOH (Table 4).

Rheological studies [8]

The viscosity of the instilled formulation is an important factor in determining residence time of drug in the eye. The prepared solutions were allowed to gel in the STF, and then the viscosity determination was carried out using Brookfield viscometer DV-II+PRO model, angular velocity range from 1 to 100 rpm. Viscosity of the formulations increased with increase in polymer concentration. The hierarchy of shear rate was reversed and an average of three readings was used to calculate viscosity (Tables 5 and 6 and Figs. 8 and 9).

Sterility testing [8,9]

Sterility testing is intended for detecting that the presence of a viable form of microorganisms and was performed for aerobic and anaerobic bacteria and fungi using fluid thioglycolate medium and soybean casein digest medium, respectively, as per the Indian Pharmacopoeia (Table 7).

Drug content analysis [7,8]

Table 1c: Parameters of ANOVA (b)

Factor	Coefficient estimate	df	Standard error	95% CI low	95% CI high	VIF
Intercept	89.24	1	0.46	88.10	90.38	
A-gelrite	−7.60	1	0.57	−8.99	−6.21	1.00
B-sodium alginate	−3.44	1	0.57	−4.83	−2.05	1.00

CI: Confidence interval, VIF: Variance inflation factor, ANOVA: Analysis of variance

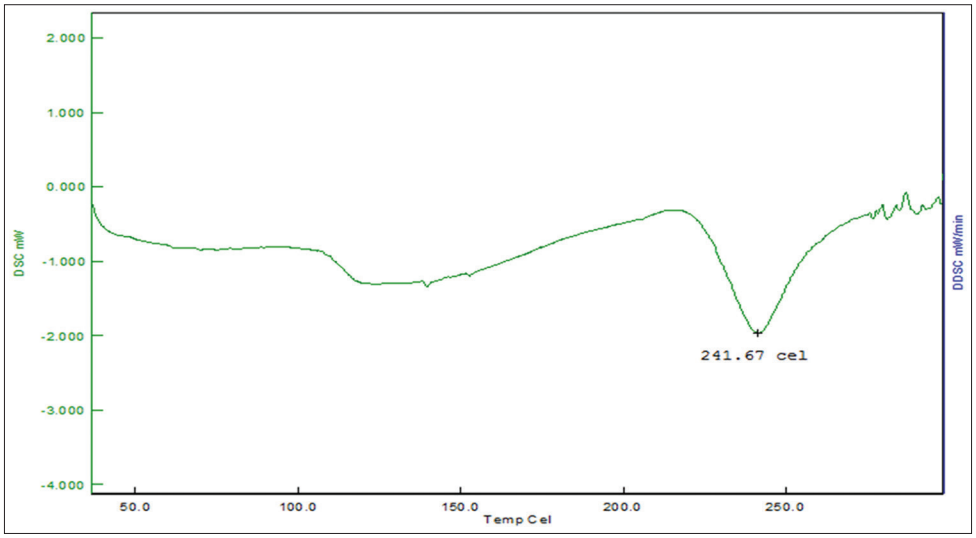

Fig. 5: Thermal analysis of a physical mixture of moxifloxacin+gelrite+sodium alginate

Estimation of MOX by spectrophotometric method

A simple and rapid method for estimation of MOX by UV spectrophotometric method was developed in STF. MOX in STF of pH 7.4 shows absorbance maxima (λ_{max}) at 288.5 nm (Table 8 and Fig. 10).

Table 2: Amount of variables in 3^2 factorial design batches

Coded values	Actual values (% w/v)	
	X1	X2
−1	0.08	0.6
0	0.1	0.8
+1	0.12	1.0

In vitro release studies [8,10]

In vitro drug release from the formulations was studied by the diffusion cell. Here, the pH of the lacrimal fluid and the blinking rate of the eye were taken into consideration and were simulated. The procedure for standard calibration is the same as mentioned under drug content determination.

Comparative evaluation of marketed product with prepared in situ gels

In vitro release studies of the marketed formulation were carried out using bi-chambered donor receiver compartment model (Franz diffusion cell) using cellophane membrane soaked overnight in the receptor medium (STF pH 7.4). The diffusion medium was 12 ml

Table 3: Contents of formulation

S. No	Ingredients	Concentration (% w/v)								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	MOX HCL	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2	Gelrite	0.08	0.08	0.08	0.1	0.1	0.1	0.12	0.12	0.12
3	Sodium alginate	0.6	0.8	1	0.6	0.8	1	0.6	0.8	1
4	Propylene glycol	8	8	8	8	8	8	8	8	8
5	Sodium chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
6	0.1 N HCl	Quantity sufficient to adjust to pH 4.4								
7	Deionized water	Quantity sufficient to 100 ml								

MOX HCL: Moxifloxacin hydrochloride. *All quantities are expressed as % w/v

Table 4: Evaluation of visual appearance, clarity, pH, and drug content

Formulation code	Visual appearance	Clarity	pH	Gelling capacity	Drug content (%)
F1	Translucent	Clear	4.4±0.029	+	87.34±0.012
F2	Translucent	Clear	4.4±0.014	+	78.11±0.01
F3	Translucent	Clear	4.4±0.008	++	73.37±0.015
F4	Translucent	Clear	4.4±0.014	+	90.17±0.12
F5	Translucent	Clear	4.4±0.021	++	87.48±0.07
F6	Translucent	Clear	4.4±0.04	+++	81.14±0.05
F7	Translucent	Clear	4.4±0.016	+++	99.75±0.045
F8	Translucent	Clear	4.4±0.163	+++	95.93±0.024
F9	Translucent	Clear	4.4±0.014	+++	95.95±0.02

SD: Standard deviation (n±3). +Gels after a few min and dissolves rapidly, ++gelation immediate and remains for few h, +++Shows gelation immediate and remains for extended period

Table 5: Viscosity of the formulation (CPS) before gelation

Shear rate (RPM)	F1±SD	F2±SD	F3±SD	F4±SD	F5±SD	F6±SD	F7±SD	F8±SD	F9±SD
1	39.1±0.121	42.3±0.063	50.1±0.304	63.1±0.102	69.6±0.127	72.8±0.106	83.7±0.100	91.3±0.086	95.7±0.066
5	36.8±0.077	39.7±0.069	47.8±0.149	61.3±0.184	66.0±0.088	72.5±0.080	80.1±0.082	88.5±0.069	89.9±0.065
10	35.0±0.085	37.6±0.069	44.7±0.085	60.4±0.078	63.4±0.106	71.2±0.074	77.5±0.064	86.1±0.092	88.2±0.077
20	21.9±0.045	34.1±0.080	39.2±0.088	56.9±0.065	59.3±0.073	66.4±0.065	72.3±0.106	77.4±0.069	81.8±0.094
30	16.5±0.074	31.5±0.066	36.4±0.131	50.7±0.085	55.5±0.066	58.3±0.082	66.9±0.063	71.6±0.085	75.1±0.094
50	15.1±0.048	24.8±0.071	29.6±0.088	42.2±0.086	47.7±0.128	51.5±0.114	57.1±0.092	56.3±0.112	70.4±0.086
100	7.2±0.053	9.5±0.131	15.1±0.131	24.3±0.094	31.6±0.069	34.1±0.090	42.6±0.086	40.8±0.069	53.7±0.236

CPS: Custom pharma service, RPM: Revolution per min, SD: Standard deviation

Table 6: Viscosity of the formulation (CPS) before gelation

Shear rate (RPM)	F1±SD	F2±SD	F3±SD	F4±SD	F5±SD	F6±SD	F7±SD	F8±SD	F9±SD
1	823.5±0.624	860.7±0.637	910.1±0.813	970.5±0.855	1020±0.965	1095.2±0.774	1140.9±1.256	1176.2±0.941	1201.9±1.157
5	731.2±0.920	813.4±0.605	866.5±0.790	905.6±0.800	980.6±0.883	1014.8±2.593	1092.5±0.941	1098.1±0.734	1169.2±0.454
10	668.4±0.662	735.9±0.565	768.9±1.079	882.7±0.623	941.2±1.067	910.9±1.259	954.6±0.848	1012.3±0.993	1130.7±1.061
20	612.9±0.828	691.3±0.610	750.7±0.69	727.3±0.889	813.4±0.864	766±0.951	878.1±0.711	846.8±0.637	869.4±0.571
30	508.7±0.232	530.1±0.834	615.3±1.111	591.5±0.928	638.7±1.098	705.4±0.697	791.4±0.962	718.4±0.588	796.6±0.989
50	320.1±0.836	488.8±1.224	461.4±1.096	503.1±0.920	564.3±1.098	645.2±0.509	713.1±0.828	634.6±0.588	704.9±1.256
100	75.5±0.700	160.1±0.786	178.6±0.771	165.6±1.025	201.7±0.932	212.3±1.080	245.8±0.787	289.0±1.019	318.1±1.283

CPS: Custom pharma service, RPM: Revolution per min, SD: Standard deviation

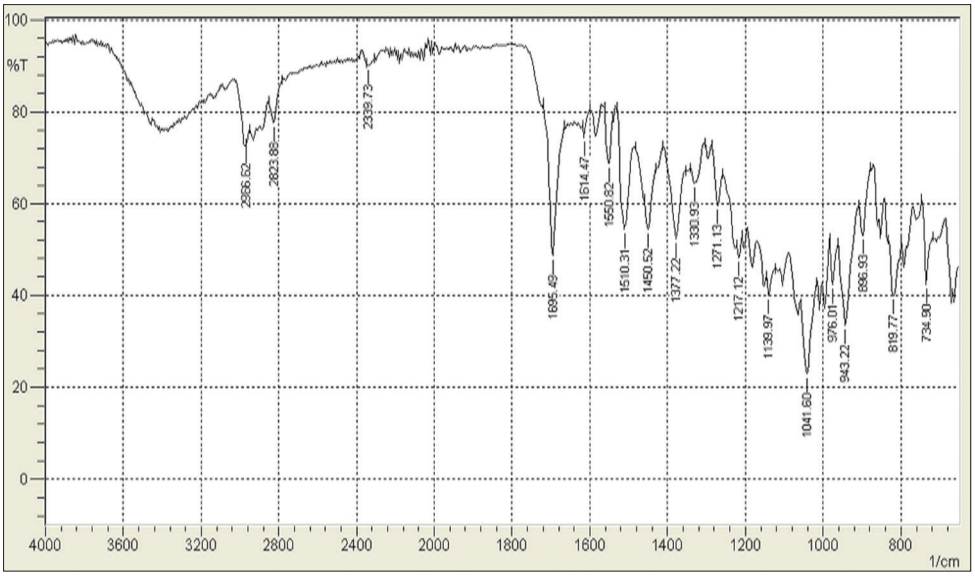

Fig. 6: Infrared spectra of *in situ* gel of moxifloxacin

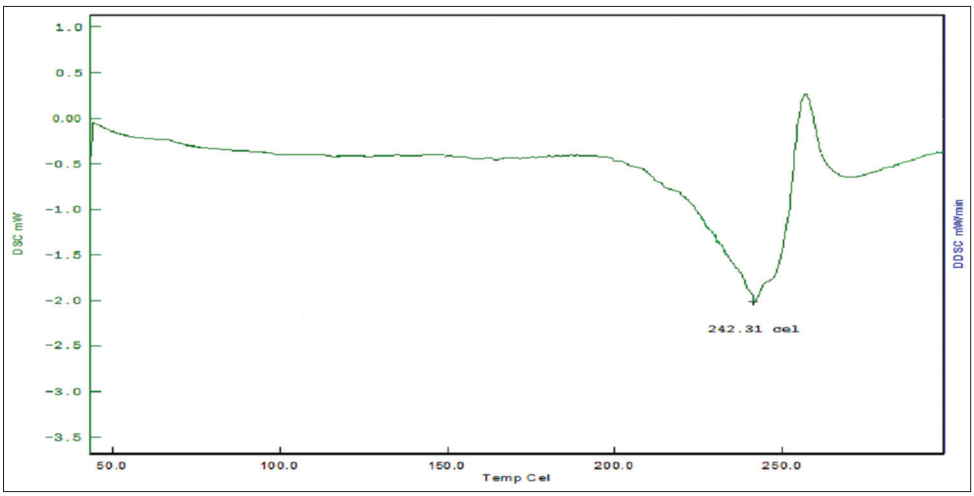

Fig. 7: Thermal analysis of moxifloxacin *in situ* gel

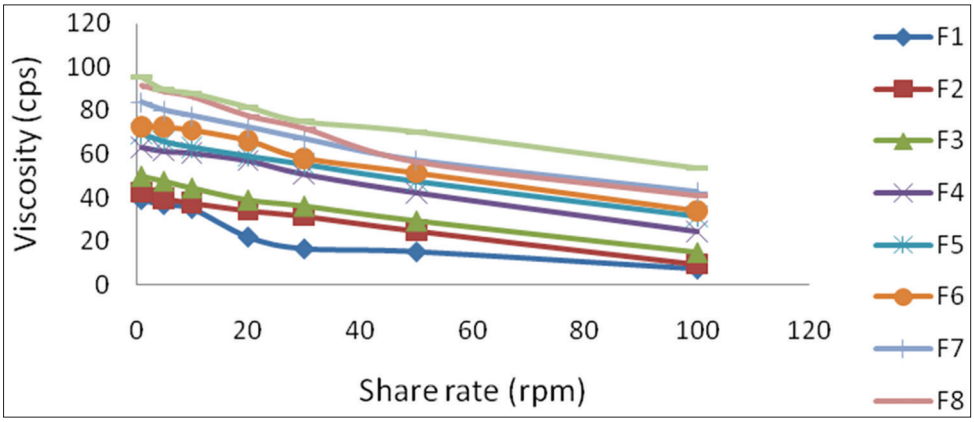

Fig. 8: Rheological studies of *in situ* gels before gelation

of STF stirred at 50 rpm at 37±0.5°C. One end of the diffusion tube was covered by a cellophane membrane. The 1 ml formulation was spread on the cellophane membrane, and membrane was placed such that it just touches the diffusion medium STF present in the receptor compartment. The drug samples were withdrawn at the interval of 1 h for the period of 8 h. From diffusion medium and analysed by a UV spectrophotometer at 288.5 nm using STF as blank (Fig. 11).

Pharmacokinetic release studies [10]

All the optimized formulations were subjected to study the release kinetics, and the best fit kinetic model was determined for the optimized formulations (Table 9).

Antimicrobial efficacy studies [11]

The antimicrobial efficacy studies were carried out to ascertain the biological activity of the optimized formulations. *Staphylococcus aureus* and *Escherichia coli* were used as the test organisms. Antimicrobial efficiency was determined by agar diffusion test employing cup-plate method. Sterile solutions of MOX (standard solution) and the developed formulations were diluted at different concentration (test solutions) these solutions were poured into cups bored into sterile nutrient agar previously seeded with test organisms (*E. coli* and *S. aureus*), after allowing diffusion of the solutions for 2 h, the agar plates were incubated at 37°C for 24 h. The zone of inhibition (ZOI) measured around each cup and was compared with that of control. The entire operation except the incubation was carried out in a laminar air flow unit. Both positive and negative controls were maintained during the study (Table 10 and Fig. 12).

Accelerated stability studies [7,9]

Stability is defined as the extent, to which a product retains within specified limits and throughout its period of storage and uses, i.e., shelf life. Stability studies were carried out on optimized formulations according to the International Conference on Harmonization (ICH) guidelines.

A sufficient quantity of formulations in previously sterilized vials was stored in desiccators containing a saturated solution of sodium chloride, which gives a relative humidity of 75±5%. The desiccators were placed in a hot air oven maintained at a temperature 40±5°C and room temperature. Samples were withdrawn at 7 days interval for 42 days. Percent drug remaining was calculated and plotted against time in days (Table 10 and Figs. 14 and 15).

Table 7: Test of sterility

Formulation code	Days of incubation						
	1	2	3	4	5	6	7
F1	-	-	-	-	-	-	-
F2	-	-	-	-	-	-	-
F3	-	-	-	-	-	-	-
F4	-	-	-	-	-	-	-
F5	-	-	-	-	-	-	-
F6	-	-	-	-	-	-	-
F7	-	-	-	-	-	-	-
F8	-	-	-	-	-	-	-
F9	-	-	-	-	-	-	-

Where "-"sign indicate the no growth. SD: Standard deviation

Table 8: Standard calibration data of MOX

S. No	Concentration (µg/ml)	Absorbance (nm)
1	0	0.000±0.000
2	2	0.2273±0.001
3	4	0.4171±0.004
4	6	0.6284±0.021
5	8	0.8471±0.011
6	10	1.0829±0.081

SD: Standard deviation (n±3), MOX: Moxifloxacin hydrochloride

RESULTS

Solubility study

The solubility of MOX was found to be dependent on pH. MOX was soluble in a cosolvent mixture of propylene glycol and water, glycerine and water 2.4 g of MOX soluble in 100 ml water.

Melting point determination

The melting point of MOX was found to be approximately 238–242°C.

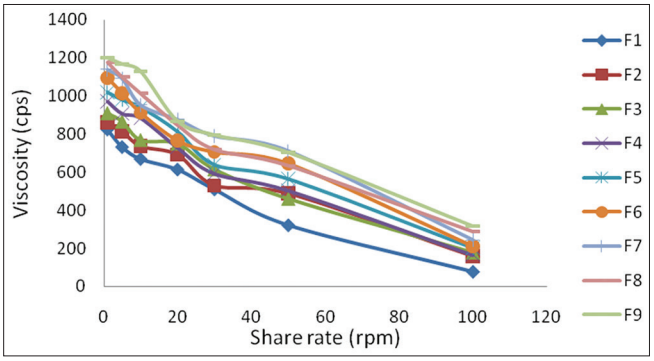

Fig. 9: Rheological studies of *in situ* gels after gelation

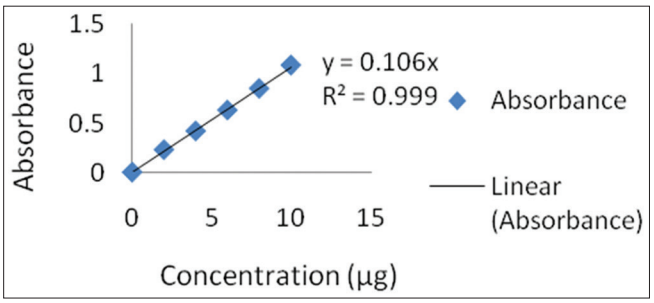

Fig. 10: Calibration curve of moxifloxacin hydrochloride in simulated tear fluid

Table 9: Regression coefficient (r^2) values of different kinetic models

Formulation	% CDR	Zero-order	First-order	Higuchi matrix	Peppas plot	
					r^2 value	"n" value
F1	98.87	0.948	0.737	0.981	0.551	0.524
F2	96.15	0.949	0.783	0.967	0.555	0.526
F3	93.68	0.933	0.931	0.993	0.541	0.500
F4	94.79	0.945	0.906	0.979	0.538	0.496
F5	91.04	0.893	0.911	0.986	0.502	0.415
F6	85.55	0.917	0.970	0.994	0.527	0.463
F7	84.05	0.879	0.965	0.980	0.485	0.474
F8	81.21	0.907	0.939	0.963	0.502	0.406
F9	77.84	0.929	0.944	0.962	0.529	0.453

CDR: ???

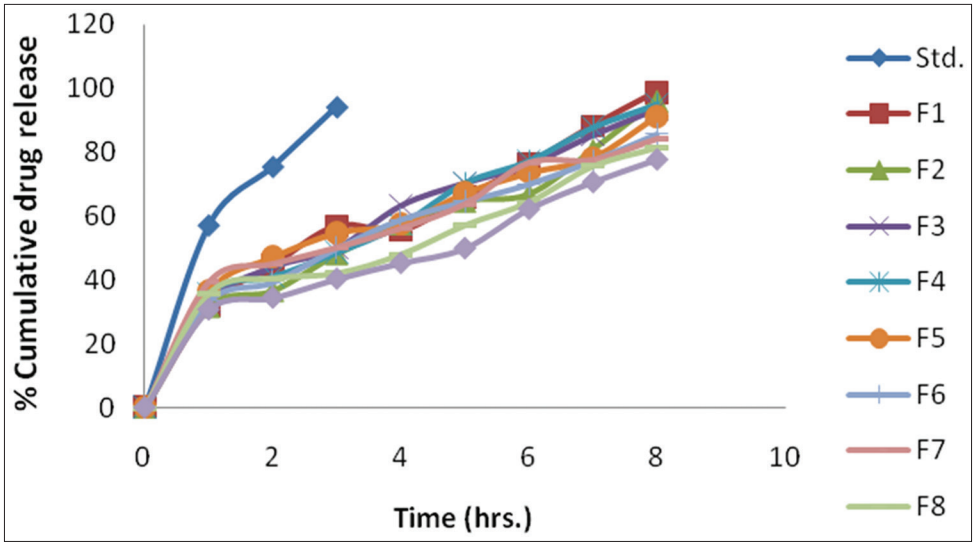

Fig. 11: Comparative *in vitro* release of marketed eye drop and *in situ* gels

Table 10: Antimicrobial activity of *in situ* gels

Test microorganisms	Diameter of the ZOI produced by *in situ* gels and marketed eye drops (Std) (mm)									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	Std
Staphylococcus aureus	48	47	46	48	48	47	48	48	47	37
Escherichia coli	53	53	52	53	52	52	53	53	53	37

ZOI: Zone of inhibition

Determination of λ_{max}/UV spectrometry

λ_{max} of MOX was found to be 288.5 in STF pH 7.4.

Identification of MOX/FT-IR spectral analysis

DSC study

Drug excipients compatibility study using

FT-IR spectroscopy study

FTIR study was carried on the pure drug, a physical mixture of drug and polymers, formulations to confirm the compatibility of the drug with other excipients used in the preparation of *in situ* gels.

DSC

Thermal characterization of pure drug and the physical mixture was performed with a calorimeter. The sample was placed in sealed aluminum pans. The samples were scanned at 20°C/min from 20°C to 300°C.

EVALUATION OF PREPARED *IN SITU* GELLING SYSTEM

Interaction studies

FTIR spectral analysis

The prepared *in situ* gelling systems were evaluated for interaction studies to ensure that there is no interaction occurred in between drug and polymers. For confirmation of stability of the drug in the prepared formulations, the IR spectra were taken and compared with that of pure drug. The result of these studies revealed that there were no definite changes obtained in the bands of the drug with respect to pure drug (Fig. 6).

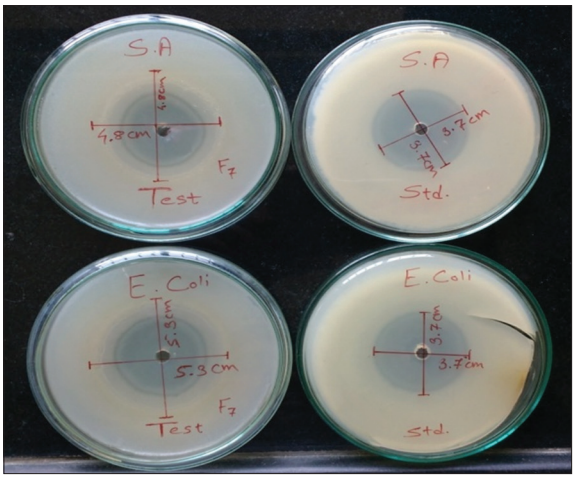

Fig. 12: Antimicrobial activity of eye drop and batch-F7

DSC analysis of MOX

Evaluation of visual appearance, clarity, pH, and drug content

All the prepared *in situ* gelling systems were evaluated for preliminary steps such as visual appearance, clarity, pH, and drug content. These formulations were translucent and clear. The pH of the formulations was found to be 4.4, and drug content was in between 73% and100% (Table 4).

Rheological studies

For the development of optimum *in situ* gelling system, two major prerequisites viscosity and gelling capacity should be taken in consideration, since the ocular shear rate is very high ranging from 0.03/S during inter-blinking periods to 4250–28500/S during blinking, viscoelastic fluid with a viscosity that is high under low shear rate condition and low under high shear rate condition, which is called pseudo-plastic fluid, is often preferred, so dynamic viscosity of formulations was measured as the change of shear rate before and after gelation (Tables 5 and 6 and Figs. 8 and 9).

Sterility testing

All the prepared *in situ* gelling systems were evaluated for the sterility. After 7 days of incubation, the results showed no microbial growth in all formulations (Table 7).

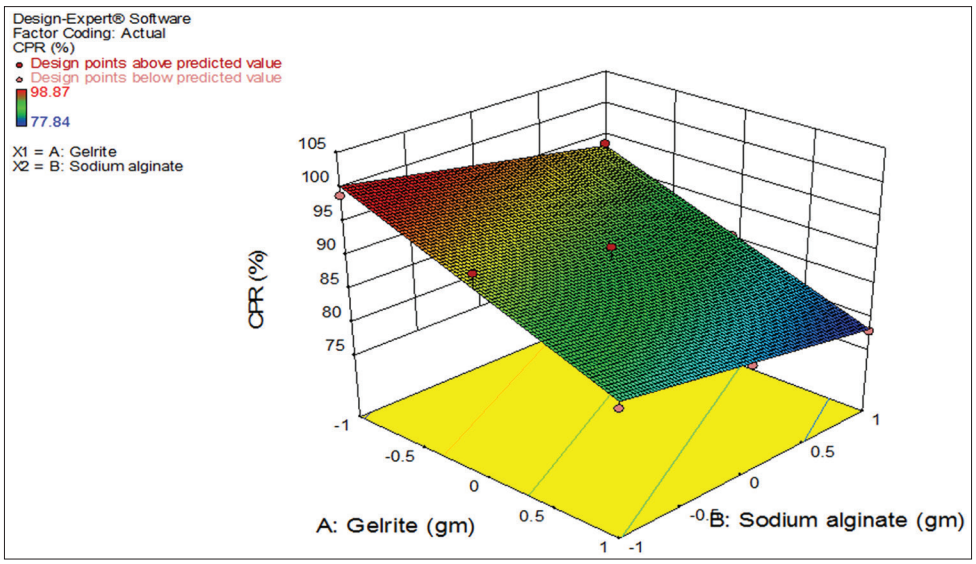

Fig. 13: Factorial plot

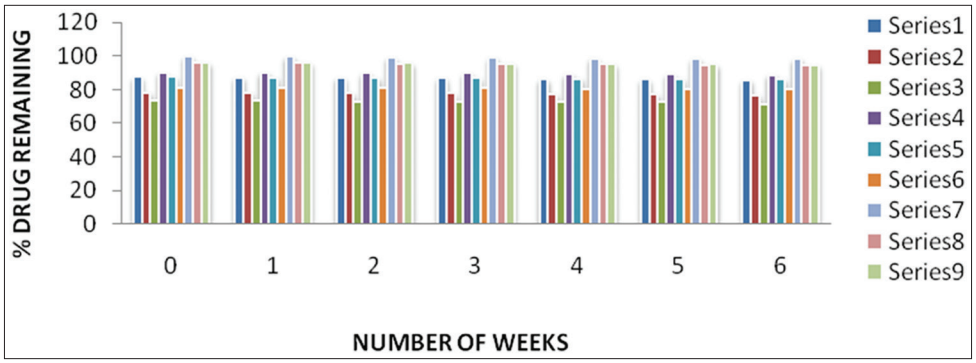

Fig. 14: Stability studies of *in situ* gels art room temperature

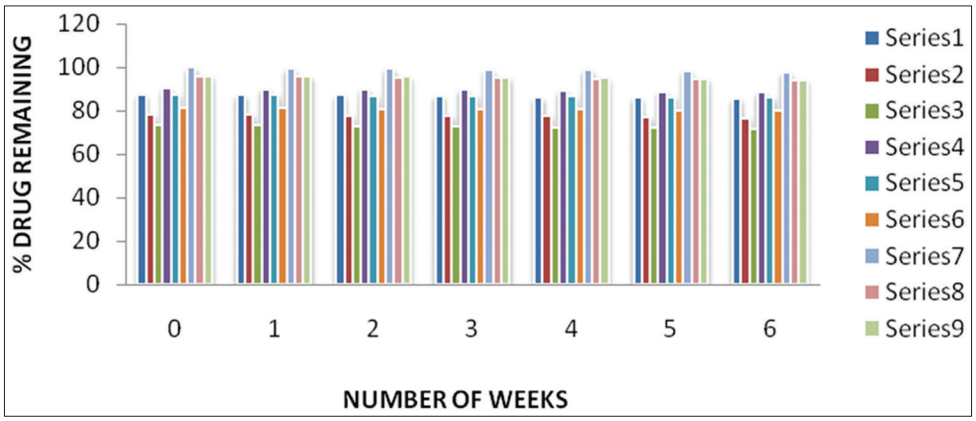

Fig. 15: Stability studies of *in situ* gels at 40°C

Estimation of MOX by spectrophotometric method

A simple spectrophotometric method for estimation of MOX was developed in STF, which exhibited λ_{max} at 288.5 nm. Results are shown in Table 8 and Fig. 10.

***In vitro* release studies**

The *in vitro* release of MOX from the prepared formulations was studied through cellophane membrane using diffusion cell. The release studies of prepared *in situ* gelling systems were carried out up to 8 h (Fig. 11).

Antimicrobial efficacy studies

The optimized *in situ* gelling formulations showed antimicrobial activity when tested microbiologically by the cup-plate technique. Clear zones of inhibition were obtained in all the formulations. The diameter of ZOI produced by formulations against all test microorganisms is given in Table 10 and Fig. 12.

Final equation in terms of actual factors:

% drug release=+89.24-7.60*gelrite-3.44*sodium alginate

Table 11: Stability studies of formulation F7

S. No	Number of weeks	Visual appearance		Clarity pH			
		RT	40°C	RT	40°C	RT	40°C
1	0	Translucent	Translucent	Clear	Clear	4.40±0.016	4.40±0.014
2	1	Translucent	Translucent	Clear	Clear	4.41±0.016	4.42±0.014
3	2	Translucent	Translucent	Clear	Clear	4.44±0.014	4.47±0.008
4	3	Translucent	Translucent	Clear	Clear	4.42±0.008	4.43±0.021
5	4	Translucent	Translucent	Clear	Clear	4.44±0.016	4.48±0.016
6	5	Translucent	Translucent	Clear	Clear	4.46±0.047	4.50±0.020
7	6	Translucent	Translucent	Clear	Clear	4.46±0.018	4.53±0.018

SD=Standard deviation (n±3)

Accelerated stability studies

According to the ICH guideline, the accelerated stability studies were carried for prepared *in situ* gelling systems. All the formulations were analyzed for visual appearance, clarity, pH, and drug remaining. 6 weeks of stability studies reveal that there was no change in visual appearance and clarity. All the formulations showed slight changes in pH, but it was in acceptable limits (±0.5). Study of percentage drug remaining in all formulations reveals that there were no definite changes observed to justify for drug degradation (Table 11 and Figs. 14 and 15).

DISCUSSION

Optimized formulations F6 (0.1% gelrite and 1.0% sodium alginate), F7 (0.12% gelrite and 0.6% sodium alginate), and F8 (0.12% gelrite and 0.8% sodium alginate) and were liquid before instillation in to eye and underwent rapid gelation on instillation in to eye and had given 85.55%, 84.05%, and 81.21% percentage cumulative drug release, respectively, the formulations were found to be clear, having good *in situ* gelling capacity, and having drug content 81–100%, optimized formulations were sterile and showed sustained drug release over 8 h period as compared to marketed eye drop, release kinetic study showed that the formulation followed Higuchi model diffusion controlled and non-Fickian release mechanism, the optimized formulations were having good antibacterial efficacy. As per the ICH guidelines, the stability study of formulations was carried out results showed that formulations were stable (translucent and clear) at room temperature as well as at 40°C.

Hence from the above results, we can conclude that F7 (0.12% gelrite and 0.6% sodium alginate) is the best formula (percentage cumulative drug release over 84.05%) and it is possible to formulate *in situ* ophthalmic gels of MOX using gelrite with sodium alginate in combination for the treatment of various bacterial infections.

CONCLUSION

The present work was carried out to develop ion activated *in situ* gel of MOX, a broad spectrum antibacterial agent used in the treatment of ocular infections, was successfully formulated as *in situ* gel-forming eye drops using gelrite as a gelling agent in combination with sodium alginate as a viscosity enhancing agent. Thus, the developed formulation is a viable alternative to conventional eye drops of its ability to sustain drug release. Furthermore, important is the ease of administration afforded and decreased frequency of administration resulting in better patient acceptance.

AUTHERS' CONTRIBUTIONS

Mr. Asish Dev conceived of the presented idea. I developed the theory and performed the computations. Mr. Asish Dev verified the analytical methods. All authors discussed the results and contributed to the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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