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 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 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 eye.

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

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-polymers such as carboxymethyl cellulose, xyloliguan.
- Temperature dependent - poloxamers (pluronic and tetronics), cellulose derivatives (methylcellulose and hydroxypropyl methylcellulose), xyloliguan.
- Ion-activated induced - alginites and gelrite (gelan 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 turnover 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 viscosity enhancing 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.
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 3² 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 3² 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](image1)

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±9.24 |
| 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    |
| AIC                              | 38.6     |

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 to 100 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]

![Fig. 5: Thermal analysis of a physical mixture of moxifloxacin+gelrite+sodium alginate](image)
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 ($
\lambda_{max}$) at 288.5 nm (Table 8 and Fig. 10).

Table 2: Amount of variables in 3$^3$ 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 ± SD               |
| 1     | MOX HCL              | 0.5                   |
| 2     | Gelrite              | 0.08                  |
| 3     | Sodium alginate      | 0.6                   |
| 4     | Propylene glycol     | 8.8                   |
| 5     | Sodium chloride      | 0.5                   |
| 6     | 0.1 N HCl            | Quantity sufficient to adjust to pH 4.4 |
| 7     | Deionized water      | Quantity sufficient to 100 ml |

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

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| +                | 87.3±0.012       |
| F2               | Translucent       | Clear   | 4.4±0.014| +                | 78.1±0.011       |
| F3               | Translucent       | Clear   | 4.4±0.008| ++               | 73.7±0.015       |
| F4               | Translucent       | Clear   | 4.4±0.014| +                | 90.7±0.012       |
| F5               | Translucent       | Clear   | 4.4±0.021| ++               | 87.4±0.07        |
| F6               | Translucent       | Clear   | 4.4±0.04 | +++              | 81.1±0.05        |
| F7               | Translucent       | Clear   | 4.4±0.016| +++              | 99.7±0.045       |
| F8               | Translucent       | Clear   | 4.4±0.163| +++              | 95.9±0.024       |
| F9               | Translucent       | Clear   | 4.4±0.014| +++              | 95.9±0.02        |

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| 47.6±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| 71.6±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| 66.0±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| 1149.0±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| 980.8±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±0.976| 910.9±1.259| 956.4±0.848| 1012.3±0.993| 1130.7±1.061|
| 20               | 612.9±0.828| 691.3±0.610| 750.7±0.690| 727.3±0.889| 813.4±0.864| 766.9±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±0.984| 645.2±0.509| 731.1±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.
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).

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.

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                     | 0.871±0.011     |
| 6     | 10                    | 1.0829±0.081    |

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

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

| Formulation | % CDR | Zero-order | First-order | Higuchi matrix | Peppas plot |
|-------------|-------|------------|-------------|----------------|-------------|
| F1          | 98.87 | 0.948      | 0.737       | 0.981          | 0.551       |
| F2          | 96.15 | 0.949      | 0.783       | 0.967          | 0.555       |
| F3          | 93.68 | 0.953      | 0.931       | 0.993          | 0.541       |
| F4          | 94.79 | 0.945      | 0.906       | 0.979          | 0.538       |
| F5          | 91.04 | 0.893      | 0.911       | 0.986          | 0.502       |
| F6          | 85.55 | 0.917      | 0.970       | 0.994          | 0.527       |
| F7          | 84.05 | 0.679      | 0.965       | 0.980          | 0.485       |
| F8          | 81.21 | 0.907      | 0.939       | 0.963          | 0.502       |
| F9          | 77.84 | 0.929      | 0.944       | 0.962          | 0.529       |

CDR: ???
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  | 47  | 48  | 48  | 47  | 37  |
| Escherichia coli        | 53  | 53  | 52  | 53  | 53  | 53  | 53  | 53  | 57  |

ZOI: Zone of inhibition

Determination of $\lambda_{max}$/UV spectrometry

$\lambda_{max}$ 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).

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% and 100% (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).
Estimation of MOX by spectrophotometric method
A simple spectrophotometric method for estimation of MOX was developed in STE, which exhibited λ<sub>max</sub> 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:
\[
\% \text{ drug release} = 89.24 - 7.60 \times \text{gelrite} - 3.44 \times \text{sodium alginate}
\]
**Table 11: Stability studies of formulation F7**

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

**AUTHORS’ 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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