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IN-SITU OPHTHALMIC GEL FORMING SOLUTION OF MOXIFLOXACIN HYDROCHLORIDE FOR SUSTAINED OCULAR DELIVERY

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Keywords:

In situ gel, Moxifloxacin Hydrochloride, Sodium Alginate (Kelton®), Hydroxypropyl methyl cellulose

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ABSTRACT: Human eye is a challenging subject for topical administration of the drugs because of its peculiar anatomical arrangements of surface tissue and impermeability of the cornea. Topical instillation of drugs through eye drops is the most important and well-accepted route of administration for the treatment of various eye disorders. Conventional ophthalmic drug delivery systems often result in poor bioavailability and therefore poor therapeutic response. Several new preparations have been developed to prolong the contact time of the medicament on the ocular surface. Successful results have been obtained with inserts and collagen shields. However, these preparations have some disadvantages, such as poor patient compliance, especially by geriatric patients. This problem can be overcome by using in situ gel forming systems of polymers that exhibit reversible phase transition. Such system can be formulated as eye drops suitable for administration by instillation into the eye, which upon exposure to the eye converts to the gel phase. The advantage of these formulations is that unlike inserts and films they do not require sophisticated equipments for manufacture and they are easily scalable. The objective of the present study was to develop an ion activated in situ gelling system for Moxifloxacin Hydrochloride, so as to increase the precorneal residence time, reduced dosing frequency and improved patient compliance. In situ gel forming solution of Moxifloxacin Hydrochloride was developed using Sodium Alginate (Keltone LVCR, Protanal®) as the gelling agent in combination with Hydroxypropyl Methylcellulose (HPMC)- Methocel E50LV which acted as a viscosity-enhancing agent. The prepared formulations were evaluated for pH , gelling capacity, drug content, in vitro diffusion studies, ex vivo diffusion studies, bioadhesion test, sterility and antimicrobial efficacy studies. **Key Findings:** The rheological behaviors of all formulations consisting of gelling polymer were not found to be affected by the incorporation of drug and sterilization. The developed formulation providedabout90% *in vitro* release and 85% *ex-vivo* release in 12 hours. Results of the present study indicate that Moxifloxacin Hydrochloride retained its antimicrobial efficacy when formulated as an *in situ* gelling system. **Conclusion:** *In situ* gel forming solutions developed for Moxifloxacin Hydrochloride using polymers like Sodium alginate and HPMC prolong the release and reduce dosing frequency of the drug. Thus, the developed *in situ* gel forming solution is an effective alternative for conventional ophthalmic drug delivery systems.

INTRODUCTION: Amongst the various routes of drug delivery, the field of ophthalmic drug delivery is one of the most interesting and challenging endeavours facing a pharmaceutical scientist.

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The anatomy, physiology and biochemistry of the eye render this organ extremely impermeable to foreign substances [1, 2]. A major problem of ocular drug delivery is not the lack of efficient drugs but the attainment of their optimal concentration at the required site of action [3]. The most important and well-accepted route of administration for the treatment of various eye disorders is the topical instillation of drugs through eye drops. Conventional pharmaceutical formulations, such as solutions, suspensions and ointments have many disadvantages viz.:

- Rapid precorneal elimination due to tear turnover
- Frequent instillation
- Enzymatic metabolism
- Nasolacrimal drainage
- Conjunctival absorption
- Blurred vision
- Absence of controlled release [3, 4]

Despite these limitations, significant improvements have been made in ocular drug delivery. The main objective of the improvement is to maintain the drug in the eye cavity for a longer period of time [5]. Successful results have been obtained especially in geriatric patients with collagen shields and inserts, inspite of disadvantages like poor patient compliance and losing the device without noticing it [6].

A more acceptable dosage form would be one that can be delivered as less frequently dosed eye drops without creating vision problems. With this in mind, recent research efforts have focused majorly on systems and technologies in which drugs can be administered as an eye drop. Major progress has been made in ophthalmic gel technology in the development of *in situ* gelling systems which are solutions administered by simple conventional instillation into the eye, changes to the gel phase upon exposure to physiological conditions thereby increasing the pre-corneal residence time of the drug enhancing the ocular bioavailability. As a result, sustained release and enhanced patient compliance is achieved.

Depending on the method employed to cause sol to gel phase transition on the ocular surface, the following three systems are recognized:

- pH-triggered -Gelling of the solution is triggered by a change in the pH by the tear fluid to 7.4. Polymer forms hydrogen bonds with mucin at higher pH which leads to formation of in-situ gel. The polymers used in this system arepseudo latex - Carbomer (Carbopol), Cellulose Acetate Phthalatelatex (CAP-latex).
- Temperature-dependent -Sustained drug delivery can be achieved by the use of a polymers like Poloxamers (Pluronic, Tetronics), cellulose derivatives (MC, HPMC), Xyloglucan that changes from solution to gel at the temperature of the eye i.e 32-34°C.
- Ion-activated - Alginates, Gelrite (Gellan gum). These polymers form a clear gel in the presence of mono or divalent cations present in the tear fluid [6].

Sodium alginate, the sodium salt of alginic acid, is a natural hydrophilic polysaccharide containing two types of monomers, β-d-mannuronic acid (M) and α-l-glucuronic acid. Alginate transforms into stable gel upon exposure to divalent cations, which is not easily eroded by tear fluid. Therefore, it might be a feasible approach to improve patient compliance to decrease the amount of alginate required for gelation by incorporating HPMC in the formulation [7].

Aim and Objective:

The objective of the present study was to develop an ion activated *in situ* gelling system for Moxifloxacin Hydrochloride, a fourth generation fluoroquinolone derivative used to treat external infections of the eye, such as acute and sub-acute conjunctivitis, bacterial keratitis and kerato conjunctivitis and to enhance patient compliance.

Moxifloxacin Hydrochloride is available under trade name Vigamox 0.5% ophthalmic solution marketed by Alcon and the usual adult dose of Moxifloxacin Hydrochloride is one drop in the affected eye thrice daily.

Since frequent dosing is required, patient compliance is compromised. Hence by formulating it as *in situ* ophthalmic gel forming solution, the dosage form would provide prolonged release of the drug over the period of 12 hours thus resulting into reduced dosing frequency and improved patient compliance.

MATERIALS AND METHODS:

Materials:

Moxifloxacin Hydrochloride was obtained from FDC Limited, Jogeshwarias a gift sample. Sodium alginate (Kelton® LVCR, which is composed of 60% mannuronic acid and 40%glucuronic acid) was kindly gifted by Signet Chemical Corporation. Hydroxy propyl methyl cellulose (MethocelE50LV, E15LV and K4M) was kindly gifted by Colorcon (UK). The other inactive ingredients viz. Sodium Chloride, Potassium Dihydrogen Orthophosphate, Sodium Hydroxide and reagents viz. Calcium Chloride, Sodium Bicarbonate were procured from Merck Chemicals.

Methods:

Preformulation studies:

Preformulation studies were commenced by characterisation of drug substance and excipients. Moxifloxacin Hydrochloride was characterised for physicochemical properties as per compendial and non compendial specifications including Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FTIR) evaluations. Drug excipient compatibility studies were conducted on a mixture of Moxifloxacin Hydrochloride and combined blend of all excipients kept in amber coloured vials for a period of 12 weeks at various conditions viz: 5°C ± 3°C, 60°C±2°C, 40°C ±2°C/ 75%RH ±5%RH. FTIR spectrum of pure drug and mixture of drug and combined blend is shown in **Fig.1** and **Fig. 2** and **Table 2.** DSC thermographs of Moxifloxacin Hydrochloride and combined blend are shown in **Fig.3** and **4**.

Analytical method development:

UV spectrophotometric method for determination of Moxifloxacin Hydrochloride at λ_{max}- 288nm was developed and standardized in Purified Water and Simulated Tear Fluid (STF) pH-7.4. The developed analytical method was employed for determination of drug content in the formulation as well as in diffusion studies. The developed analytical method was validated for parameters like linearity, accuracy, precision, recovery, repeatability. The results of the validation studies are depicted in **Table 1**.

TABLE 1: VALIDATION PARAMETERS OF DEVELOPED UV METHOD OF MOXIFLOXACIN HYDROCHLORIDE

Parameter	Results	
	In purified water	In Simulated Tear Fluid
Linearity	R^2= 0.9998	R^2 = 0.9986
Range	2-10 µg/ml	1-10 µg/ml
% Recovery	Formulation:100.98% Standard drug solution:100.13%	Formulation: 101.06% Standard drug solution:99.63%
Repeatability	%RSD= 0.25	%RSD= 0.42
Intraday precision	%RSD= 0.72	%RSD= 0.95
Interday precision	%RSD= 0.90	%RSD= 0.73

Formulation Development:

Optimization of polymer concentration in gel forming solution:

The main prerequisites of an ocular gelling system are gelling capacity (speed and extent of gelation) and viscosity. The formulation should have an optimum viscosity, which will allow its easy instillation into the eye as a liquid (drops), which will then undergo rapid sol to gel transition due to ionic interaction. Moreover, to facilitate sustained release of the drug to the ocular tissues, the *in situ* formed gel should retain its integrity for a prolonged period of time without getting eroded. Aqueous solutions of different concentrations of Sodium alginate and Hydroxypropyl methyl cellulose of different grades were Prepared (formulation codes A1, A2,.,A13) and evaluated for gelling capacity and viscosity in order to identify the compositions suitable for use as *in situ* gelling system. The formulation details are mentioned in **Table 3**.

The detailed procedure for preparing the *in situ* polymer gel-forming system is outlined below:

HPMC solutions were prepared by dispersing the required amount HPMC of various grades in sufficient volume of purified water with continuous stirring until completely dissolved. Sodium Alginate in the fixed concentration was then added to HPMC with continuous stirring until completely dissolved, and the polymer solution was subjected

to sterilization by autoclaving at 121º C and 15p.s.i. for 15 minutes. Buffer salt Potassium Dihydrogen Phosphate and tonicity adjusting agent, Sodium Chloride in fixed concentration were then added to the above solution and the solution was filtered through 0.22µm sterilizing grade Poly vinylidene difluoride hydrophilic membrane filter. The filtered solution was added to previously sterilized and cooled polymer dispersion under constant stirring until an uniform clear solution was obtained. Purified water was then added to make the volume and finally filtered through 0.45 µm membrane filter.

This exercise of optimizing polymer composition was performed prior to inclusion of drug in the gel forming solution. Polymer solutions A1 to A13 were evaluated for gelling capacity and viscosity. In addition, effect of moist heat sterilization on polymer gelling capacity and viscosity was evaluated. Drug was incorporated in those polymer compositions which yielded stable gel for a period of 8 hours and viscosity in the range of 150-300 cps before gelation and 700 – 1000 cps on contact with the simulated tear fluid as indicated in **Tables 7** and **8.** Based on the observations and results of gelling capacity and viscosity of polymer solutions, A5, A6, A8, A9, A11 and A12 were selected for incorporating drug to prepare finished pharmaceutical product. On incorporation of drug, the formulations were denoted as M5, M6, M8, M9, M11 and M12. The composition details are mentioned in **Table 4.**

Ophthalmic formulations are required to be sterile, isotonic and compatible with ocular fluid. In order to achieve these attributes, pH and isotonicity of product should be equivalent to ocular fluids. pH of the formulation is required to be maintained for improving compatibility of formulation as well for maintaining stability of the drug and preservative.

An ophthalmic formulation should be ideally buffered to a pH of 6.5 to 7.5, as the normal physiological pH of tear fluid is 7.4. The pH values of ophthalmic solutions should be adjusted within the range to provide an acceptable shelf life. They are buffered adequately to maintain stability within the range for at least 2 years.

The buffer capacity should be adequate for stability of the formulation yet low enough so that the overall pH of the tear fluid is disrupted only momentarily on instillation of formulation and the physiological pH is brought back to normal value instantaneously. Based on drug solubility and pH, Potassium Dihydrogen Orthophosphate was selected as buffer salt, the pH was adjusted to 6.8 using Sodium Hydroxide. The quantity of buffer included in the formulation was computed on the basis of required value of pH and tonicity.

An ophthalmic formulation is isotonic when the magnitudes of the colligative properties of the solution are equal to that of physiological fluid. Eye can tolerate a normal osmolarity range of 260-320 mOsmol /litre. The quantity of tonicity adjusting agent added in the formulation was decided on the basis of osmolarity contributed by each ingredient of the formulations.

The gelling polymers at specified concentrations in the formulations did not contribute appreciably to tonicity values. Buffer salt in a specific concentration used in the formulation contributed an osmolarity of 122.3 mOsmol/litre. Moxifloxacin Hydrochloride at specified label claim of 0.5% w/v contributed an osmolarity of 11.4 mOsmol /litre .Hence, to adjust the osmolarity within normal range, 0.5% of sodium chloride was added, that contributed an osmolarity value of 170.9 mOsmol/litre. The total osmolarity of Moxifloxacin Hydrochloride formulation amounted to 316.0 mOsmol/litre.

In these formulations, preservative was not included as the drug itself forms a self-preserving system. Even the marketed formulation does not contain preservative.

The gelling capacity was determined by placing a drop of the formulation in a watch glass containing 2mL of artificial tear fluid freshly prepared and equilibrated at 37°C and visually assessing the gel formation and noting the time for gelation and the time for which intact gel was observed and time taken for the gel formed to dissolve as shown in **Fig.5** to **7.** The composition of artificial tear fluid was sodium chloride 0.670 % w/v, sodium bicarbonate 0.20% w/v, calcium chloride dihydrate

0.008%w/v dissolved in purified water. Viscosity of the prepared formulations (A1 to A13) were determined using Brookfield Viscometer. To evaluate the viscosity change after instillation and mixing with simulated tear fluid (STF), rheological measurements were taken before and after diluting the formulations (A1, A..,A13) with simulated tear fluid in 25:7 ratio [8, 9].

Evaluation of the formulation:

The prepared formulations were evaluated for pH, drug content, and viscosity determination using Brookfield viscometer. Effect of sterilization and drug incorporation on polymer viscosity was determined. The findings of rheological studies are depicted in **Fig.8**. Selected formulations were evaluated for bioadhesion studies and in vitro and ex vivo drug diffusion studies

In addition to these drop volume was determined. Drop volume is evaluated to determine the amount of the drug that gets instilled when 1 drop of the formulation is instilled in the eye cavity.

Bioadhesion after gel formation:

Goat cornea was used for this study. One drop of developed formulation was added to the corneum side which forms gel on exposure to cornea. Then this glass slide was kept on a glass beaker containing STF in such a way that the corneum side is in continuous contact with STF **(Fig.14**). STF was stirred continuously on magnetic stirrer. The gel formed was then observed visually for its bioadhesion at various time intervals [10].This intact nature of gel adhering to ocular tissue was assessed by adding a water soluble dye to the formulation. If the dye remained localized, it indicated that the gel was intact and adhered to the mucosa.

***In-vitro* drug diffusion study:**

In-vitro drug release studies of the formulations were conducted and compared with conventional marketed formulation using Franz diffusion cell apparatus. Dialysis membrane- 150 (Hi media) previously soaked overnight in the receptor medium was used to study the *in-vitro* diffusion of the formulations. The receptor chamber was filled with freshly prepared STF pH 7.4. The donor compartment was loaded with 1ml of formulation. Aliquots of receptor medium were withdrawn and replenished with fresh medium at specific intervals of time and analyzed for drug content [11, 12].

***Ex vivo* corneal permeation using goat cornea:**

Goat cornea was used for the present investigation to study the permeation across the corneal membrane. Whole eyeballs of goat were procured from a slaughter house and kept in normal saline maintained at 4ºC. The cornea were carefully removed along with a 5–6 mm of surrounding scleral tissue and washed with cold saline.

The washed corneas were kept in cold freshly prepared STF, pH 7.4. The study was carried out by using Franz-diffusion cell in such a way that corneum side continuously remained in an intimate contact with formulation in the donor compartment. The receptor compartment was filled with STF pH 7.4 at 37º C ± 0.5º C. 1 ml of developed formulation was placed on the epithelial surface of each cornea in the donor chamber and covered with glass lids.The samples were withdrawn at different time intervals and analyzed for amount of drug diffused [13, 14, 15].

Release Kinetics and Diffusion Mechanism from optimized in situ gelling formulations:

The results obtained of *ex vivo* diffusion studies were fitted into various mathematical models as follows:

- Cumulative percent drug released vs. Time (Zero order rate kinetics).
- Log Cumulative percent drug retained vs. Time (First order rate kinetics).
- Cumulative percent released vs. T [Higuchi's classical diffusion equation (Higuchi matrix)].
- Log of cumulative percent drug released vs. Log Time (Peppas exponential equation).
- (Percentage Retained)$^{1/3}$vs. Time (Hixson-Crowell erosion equation).

The order of drug release kinetics was predicted by computing Regression coefficient of the curves and values obtained for "R" are as depicted in **Table 6**.

Sterility:
Sterility test was performed as per the compendial method. Direct inoculation method was used. 2ml of the formulation was aseptically transferred to fluid thioglycollate medium (20 ml) and soyabean-casein digest medium (20 ml) separately. The liquid was mixed with the media. The inoculated media were incubated for 14 days at 30ºC to 35ºC in the case of fluid thioglycollate medium and 20ºC to 25ºC in the case of soyabean-casein digest medium. Both positive and negative controls were maintained throughout the study [16].

Antimicrobial efficacy studies:
This was determined by the agar diffusion test employing 'cup plate technique' for measuring the zone of inhibition. Sterile solution of Moxifloxacin Hydrochloride in phosphate buffer, pH 6.8 (standard solution), Marketed formulation and the developed formulation diluted suitably with phosphate buffer, pH 6.8 (test solution) were poured into cups bored into sterile nutrient agar previously seeded with test organisms (*Pseudomonas aeruginosa* and *Staphylococcus aureus*). These microorganisms were chosen for the study since they are prominent bacteria that cause ocular infections like corneal ulceration, keratitis, endophthalmitis which lead to permanent loss of vision if untreated.

After allowing diffusion of the solutions for 2hours, the agar plates were incubated at 37°C for 24 hrs. The zone of inhibition (ZOI) measured around each cup was compared with that of control [4, 17].

Stability studies:
Stability studies were carried out on optimized formulations according to International Conference on Harmonization (ICHQ1 A R2) guidelines. The formulations were stored at 5°C ± 3°C (control), 25°C±2°C/60%RH±5%RH and accelerated stability storage condition (40°C ±2°C / 75%RH ±5%RH) for a period of three months. The formulations were evaluated every month for appearance, pH, gelling capacity, viscosity and drug content and after three months for *ex vivo* permeation. In addition to stability studies, the optimized formulation was subjected to Freeze thaw or Temperature cycling studies.

RESULTS:
Preformulation Studies:
Preformulation compatibility study results showed that there was no interaction observed between Active Pharmaceutical Ingredient(API) and excipients. No change in the visual appearance of physical mixtures of Moxifloxacin Hydrochloride with excipients was observed at the end of 12 weeks. The percent content of Moxifloxacin Hydrochloride was found to be in the range of 98.39% to 102.22%.

From the spectral study it was observed that there was no change in characteristic bands of Moxifloxacin Hydrochloride under stressed conditions and in presence of excipients thus no chemical interaction was observed between Moxifloxacin Hydrochloride and excipients. The IR spectra of the drug and the excipients showed similar peaks for functional groups. The prominent peaks characteristic of functional groups like –F, -C=O, -NH stretching, -OH and aromatic substitution were retained at characteristic frequency values in pure drug as well as in excipient blend as indicated in **Table 2** and **Fig.1** and **2.**

TABLE 2: REPORTED AND OBSERVED IR FREQUENCY OF MOXIFLOXACIN HYDROCHLORIDE AND ITS COMBINED BLEND

Functional group	Reported frequency (in cm^{-1})	Observed frequency in pure drug (in cm^{-1})	Observed frequency in combined blend (in cm^{-1})
-F	1091	1091	1090
-C=O	1698	1698	1698
-NH stretching	3467	3466	3468
O-H	3527	3528	3527
Aromatic substitution	788	788	786

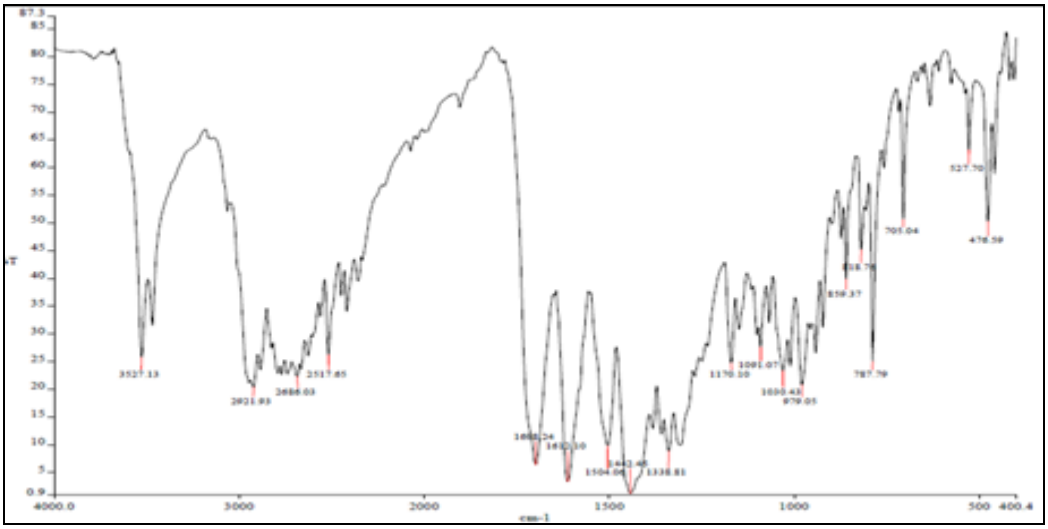

FIG. 1: IR SPECTRUM OF MOXIFLOXACIN HYDROCHLORIDE

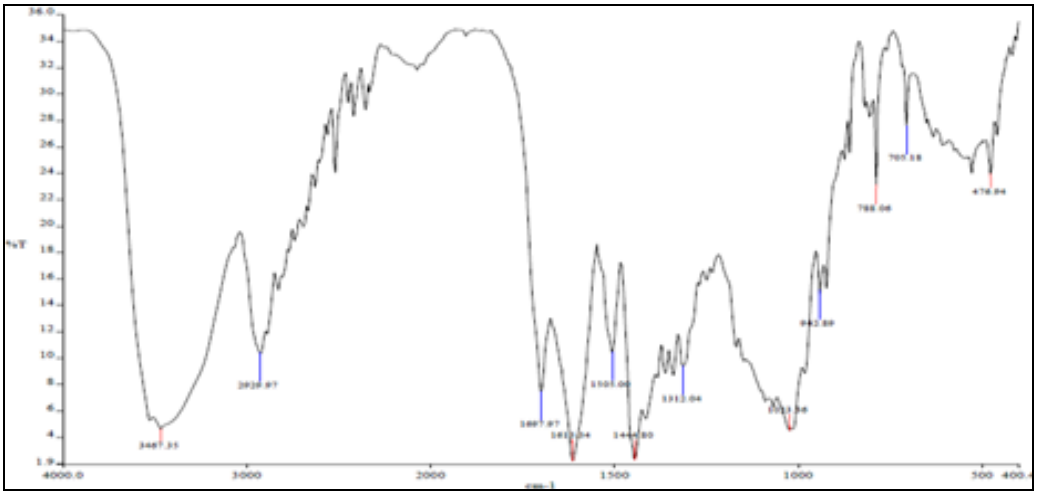

FIG. 2: FTIR SPECTRUM OF MOXIFLOXACIN HYDROCHLORIDE WITH COMBINED BLEND OF EXCIPIENTS

Compatibility of the drug with the added excipients and polymers was assessed by Differential Scanning Calorimetry (DSC). DSC thermographs were taken using Mettler equipment under nitrogen atmosphere. Graphs were reported at the temperature between 30°C to 300°C at the rate of 10°C / minute for the pure drug and drug with excipient blend.

An endothermic transition assignable to the melting of the compound was observed at a temperature of 249-250 ^{0}C which was observed for the drug as well as for drug excipient blend.

Moxifloxacin Hydrochloride showed awell characterized and recognized endothermic peak at the temperature of 249.3ºC (**Fig. 3**). Moxifloxacin Hydrochloride in the excipient blend exhibited apeak at 250.2°C (**Fig.4**)

Miniscule changes were observed in the peak temperature. Slight changes in the melting endotherm of the drug could be due to the mixing of the drug and excipients, and may not necessarily indicate potential incompatibility [18, 19, 20].

The thermographs of pure drug as well as drug in excipient blend were found to be similar. Compatibility of the drug with the excipients was maintained.

Formulation development:

Optimization of polymer concentration in gel forming solution.

Formulation A1, A3 and A7 showed the formation of gel within a 1-2 minutes which eventually collapsed and dissolved rapidly as the dye spread quickly in the STF. Formulation A2, A4, A8 and

A11 showed immediate gelation and gel consistency was maintained for 3 to 6 hours, whereas the formulations A5, A6, A9, A10, A12 and A13 showed immediate gelation. It was observed that there was no spreading or diffusion of the dye from the gel which indicated formation of stable gel structure as shown in **Fig.5** to **7**. The gelation may be due to ionic cross linking of the alginate chains by the divalent cations present in the STF.

Table 3 depicts viscosity values obtained for formulations A1 to A13. All the formulations were liquid at room temperature and underwent rapid gelation upon contact with STF. In spite, of exhibiting higher values for viscosity, Polymer solution A9 was considered as the representative formulation for comparative evaluation with other formulations of lower viscosity values.

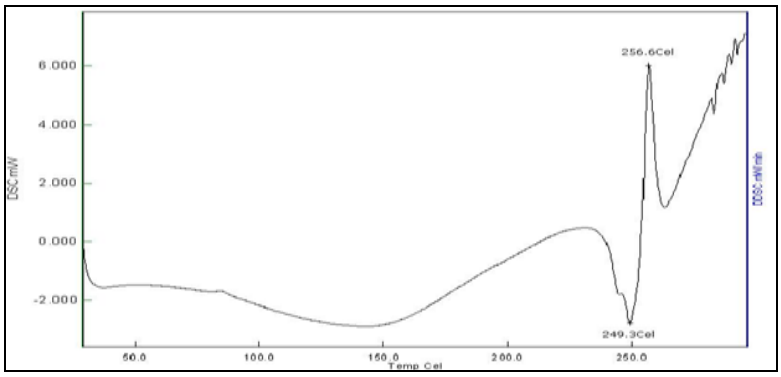

FIG. 3: DSC THERMOGRAPH OF MOXIFLOXACIN HYDROCHLORIDE

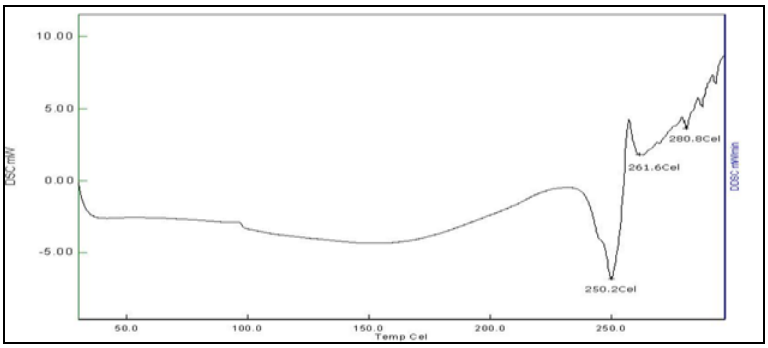

FIG. 4: DSC THERMOGRAPH OF MOXIFLOXACIN HYDROCHLORIDE WITH COMBINED BLEND OF EXCIPIENTS

TABLE 3: COMBINATIONS OF SODIUM ALGINATE AND DIFFERENT GRADES HPMC STUDIED

Formulation Code	HPMC grade	Concentration (% w/v)		Gelling Capacity	Viscosity(cps) at 5 rpm	
		HPMC	Sodium Alginate		Before Gelling	After Gelling
A1	-	-	1	+	24.5	67.8
A2	-	-	2	++	85.5	196
A3	E50 LV	0.5	1	+	52.4	155
A4	E50 LV	1	1	++	65.4	186
A5	E50 LV	1.5	1	+++	115.8	305.6
A6	E50 LV	2	1	+++	157.2	364
A7	E15LV	1	1	+	45.6	120.6
A8	E15LV	2	1	++	81.6	155.4
A9	E15LV	3	1	+++	241	390.4
A10	E15LV	4	1	+++	355.3	556
A11	K4M	0.3	1	++	103	212
A12	K4M	0.5	1	+++	172.4	293.3
A13	K4M	0.7	1	+++	258.5	435

Note: (-) No phase transition,
(+) Phase transition immediate, collapse of gel structure within 1-2 hrs,
(++) Phase transition immediate, collapse of gel structure within 3-6 hrs,
(+++) Phase transition immediate, and gel structure stable for more than 8 hrs.

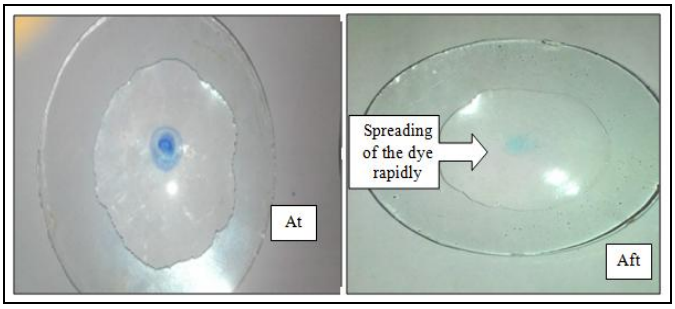

FIG.5: REPRESENTATIVE OF FORMULATIONS A1, A3 AND A7, (+) PHASE TRANSITION IMMEDIATE, COLLAPSE OF GEL STRUCTURE WITHIN 1 TO 2 HOURS

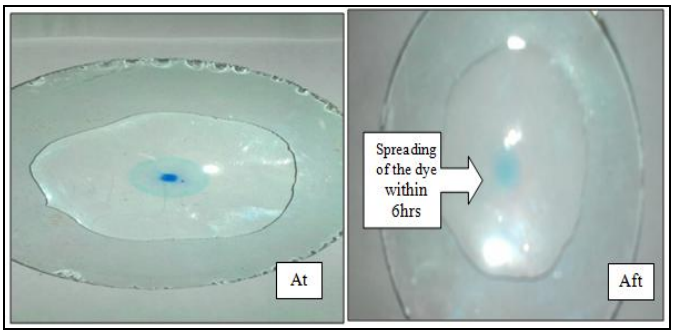

FIG.6: REPRESENTATIVE OF FORMULATIONS A2, A4, A8 AND A11, (++) PHASE TRANSITION IMMEDIATE, COLLAPSE OF GEL STRUCTURE WITHIN 3 TO 6 HOURS.

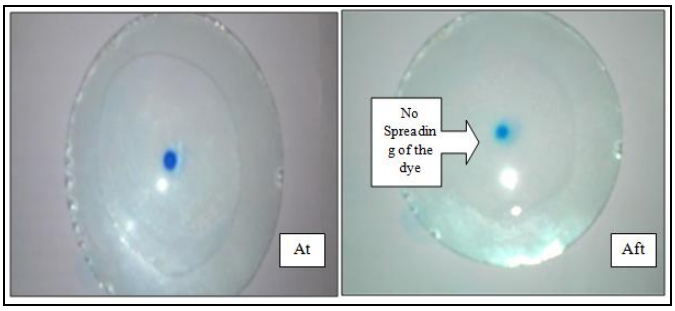

FIG.7: REPRESENTATIVE OF FORMULATIONS A5, A6, A9, A10, A12 AND A13, (+++) PHASE TRANSITION IMMEDIATE, GEL STRUCTURE STABLE FOR MORE THAN 8 HOURS

Formulations A10 and A13 showed very good gelling ability. However these formulations showed very high viscosity value in the range of 250-360 cps before gelation, and are not suitable for instillation as eye drops. Moreover, solutions with such viscosity values are not filterable through membrane filters. Therefore Formulations A10 and A13 were not considered for further formulation and evaluation.

On the basis of gelling capacity and viscosity, formulations A5, A6, A8, A9, A11 and A12 were selected for incorporating drug to prepare finished pharmaceutical product. Selected formulations after incorporation of drug were then coded as M5, M6, M8, M9, M11 and M12as indicated in **Table 4**.

TABLE 4: FORMULATION DESIGN FOR *IN SITU* GELLING SYSTEMS

Ingredients (%w/v)	M5	M6	M8	M9	M11	M12
Moxifloxacin Hydrochloride equivalent to Moxifloxacin	0.5	0.5	0.5	0.5	0.5	0.5
Sodium Alginate	1	1	1	1	1	1
HPMC E50 LV	1.5	2	-	-	-	-
HPMC E15 LV	-	-	2	3	-	-
HPMC K4M	-	-	-	-	0.3	0.5
Sodium Chloride	0.5	0.5	0.5	0.5	0.5	0.5
Potassium Dihydrogen Orthophosphate	0.68	0.68	0.68	0.68	0.68	0.68
Sodium Hydroxide	q.s. to pH 6.8	q.s. to pH 6.8	q.s. to pH 6.8	q.s. to pH 6.8	q.s. to pH 6.8	q.s. to pH 6.8
Purified Water	q.s.	q.s	q.s	q.s.	q.s.	q.s.

pH and Drug Content:

The pH of the formulations was in the range of 6.6 to 7.0. Drug content of all the formulations was found to be within specification limits as indicated in **Table 5**.

TABLE 5: CLARITY, pH AND DRUG CONTENT OF THE PREPARED *IN SITU* GELLING FORMULATIONS OF MOXIFLOXACIN HYDROCHLORIDE

Formulation Code	Clarity	pH	Drug content (%w/v)
M5	Clear	6.69	99.37
M6	Clear	6.76	101.21
M8	Clear	6.73	98.88
M9	Clear	6.70	98.16
M11	Clear	6.79	102.44
M12	Clear	6.83	102.08

Drop volume:

Volume of one drop was calculated and was found to be 0.03899 ml i .e. approximately 40µL for Moxifloxacin Gelling Formulations.

Rheological studies:

Figures8 and 9depict the viscosity values obtained for polymer solutions A1 to A13 obtained before and after gelling. A5, A6, A8, A9, A11 and A12 compositions were further selected for incorporating drug for preparation of finished pharmaceutical product. Effect of sterilization on viscosity of polymer was studied. Moreover effect of drug incorporation on overall viscosity of formulation was evaluated The results showed that there was no significant change in the viscosity of the formulations after sterilization of polymeric solution and incorporation of drug in the

formulation as depicted in **Fig.10** and **11**. The formulations were in a liquid state and exhibited low viscosity and on addition of STF the solution transformed into gels with high viscosity.

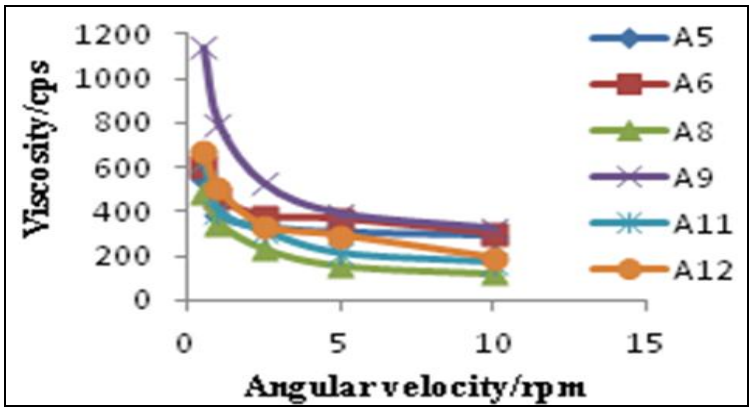

FIG.8: RHEOLOGICAL PROFILE OF FORMULATIONS BEFORE STERILIZATION AND AFTER GELLING

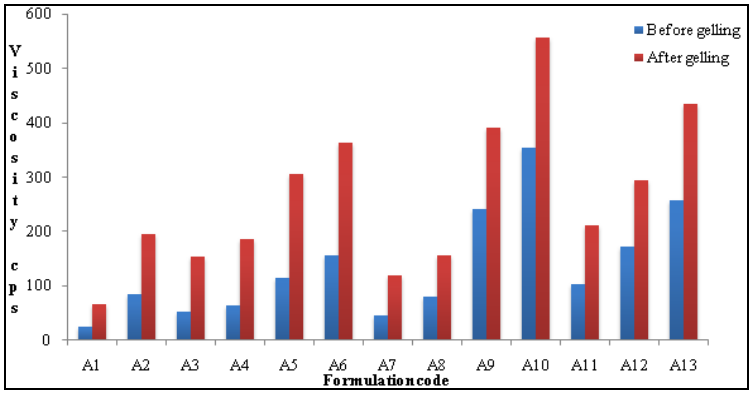

FIG.9: COMPARISON OF VISCOSITY OF THE FORMULATIONS BEFORE AND AFTER GELATION

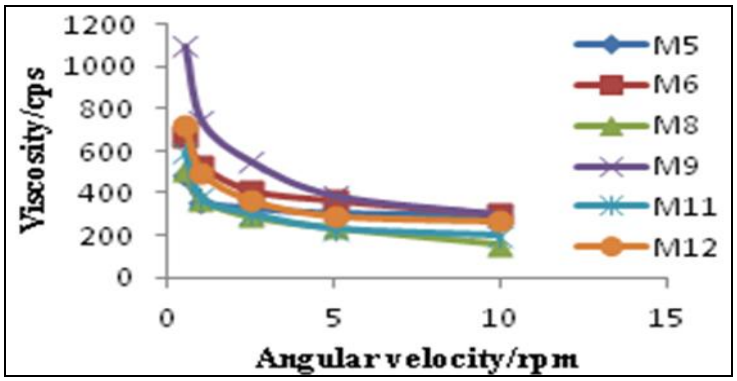

FIG. 10: RHEOLOGICAL PROFILE OF FORMULATIONS AFTER STERILIZATION, DRUG INCORPORATION AND AFTER GELLING.

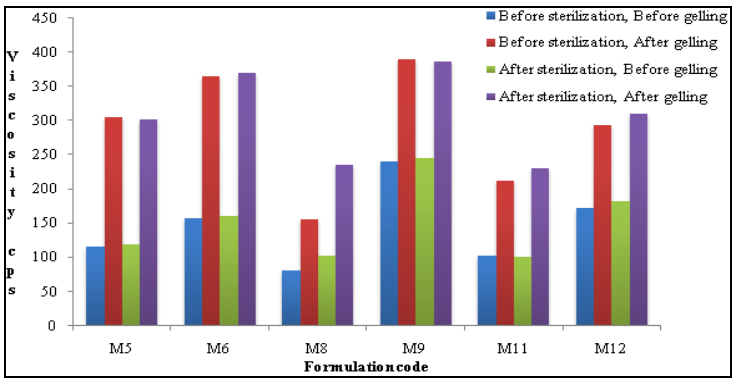

FIG.11: COMPARISON OF VISCOSITY OF THE FORMULATIONS BEFORE AND AFTER STERILIZATION AND DRUG INCORPORATION AT 5 RPM

In- vitro drug diffusion study:

The *in situ* gelling formulations viz. M5, M6, M8, M9, M11 and M12 were subjected to *in vitro* diffusion studies. Drug diffusion data obtained is shown in Figure12, which comprises of the plot of cumulative percent drug diffused as a function of time. The *in vitro* diffusion data indicated that the formulations M5, M6 and M12 showed sustained drug diffusion over a period of 12 hours when compared to other formulations. The % drug diffused from these formulations was about 90% at the end of 12 hours. Formulations M8, M9 showed % drug diffusion of about 72% to 77% after 12 hours. Prepared formulations showed prolonged release of drug as compared to conventional marketed formulation i.e. ophthalmic solution.

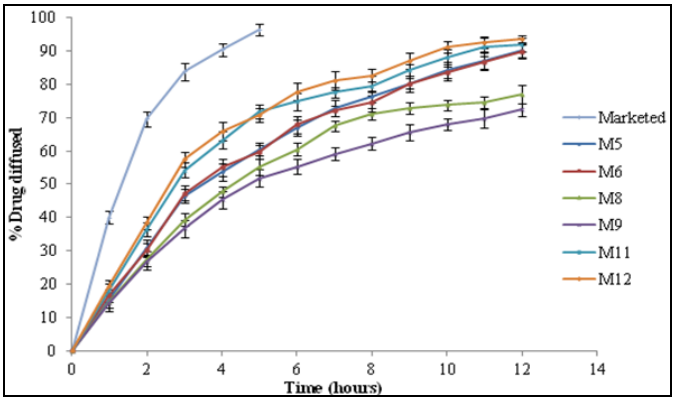

FIG. 12: *IN VITRO* PERMEATION OF MOXIFLOXACIN HYDROCHLORIDE FROM GEL FORMING FORMULATIONS

Ex vivo corneal permeation studies using goat cornea:

The *ex vivo* drug permeation for selected formulations, M6 and M12 was investigated. The *ex vivo* permeation profile indicated that the drug diffusion from the formulation M6 and M12 was 83.63% and 75.64% respectively at the end of 12 hours which showed prolonged drug release as compared to marketed formulation.(**Fig.13**) Thus formulation M6 was selected as final formulation..

Release Kinetics and Diffusion Mechanism from optimized in situ gelling formulations:

The results of statistical analysis of curve estimation were used in order to develop regression models that have the best R^2 values. Overall curve fitting showed that the permeation profiles of *in situ* gel forming solution of M6 and M12 followed the first-order model with correlation coefficients (R^2) 0.9886 and 0.9896 respectively. However since the polymer in presence of STF is forms hydrogel we can anticipate that apart from first order release kinetics, the drug may show release by the process of diffusion by Higuchi matrix model *in vivo* as depicted in **Table 6**. In addition, diffusion parameters like Flux and Permeability coefficient were calculated as depicted in **Table 7.**

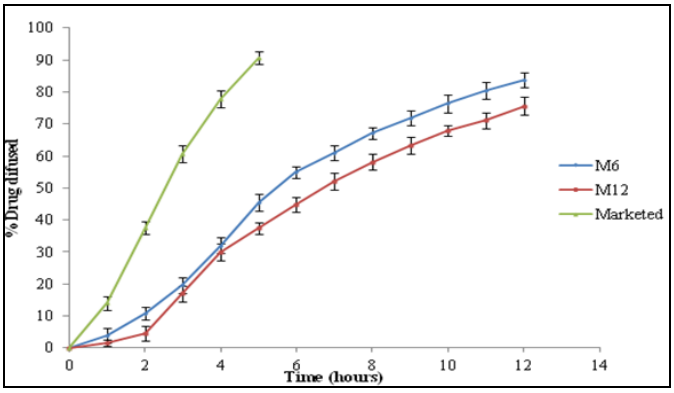

FIG.13: *EX-VIVO* PERMEATION PROFILE OF MOXIFLOXACIN HYDROCHLORIDE FROM GEL FORMING FORMULATIONS

TABLE 6: RELEASE KINETICS FROM MOXIFLOXACIN HYDROCHLORIDE *IN SITU* GELLING FORMULATIONS BASED ON VARIOUS MODELS

Permeation parameters	R^2 value	
	M6	M12
Zero order	0.9679	0.9737
First order	0.9886	0.9896
Higuchi	0.9285	0.9174
KorsmeyerPeppas	0.9704	0.9437
Hixon Crowell	0.9884	0.9890

TABLE 7: *EX -VIVO* PERMEATION DATA OF OPTIMIZED FORMULATIONS OF MOXIFLOXACIN HYDRO CHLORIDE

Batch	% drug diffused at the end of 12 hours	Flux (µg/cm^2/hr)	Permeability co-efficient (cm/hr)
	***Ex-vivo* data**		
M6	83.63%	489.62 µg/cm^2/hr	0.09792 cm/hr
M12	75.64%	445.41 µg/cm^2/hr	0.08908 cm/hr
Marketed	90.78% (At the end of 5 hours)	1226.026 µg/cm^2/hr	0.24520 cm/hr

Bioadhesion

The developed formulation exhibited satisfactory bioadhesion (**Fig.14**) indicating that the formulations when instilled in the eye will be retained in the ocular cavity. It was observed that as the concentration of polymers (Sodium Alginate and HPMC) increased the duration of bioadhesion time increased. Increase in bioadhesion time of

formulations may be due to presence of HPMC along with Sodium Alginate.

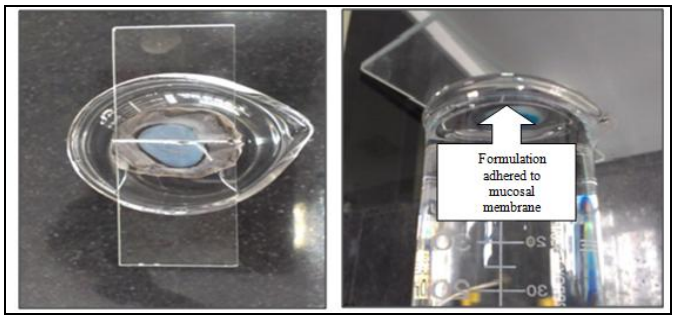

FIG. 14: BIOADHESION STUDY AFTER GEL FORMATION

Sterility:

There was no appearance of turbidity in the negative control as well as test sample and hence no evidence of microbial growth was observed. So the developed formulations being examined therefore passed the test for sterility.

Antimicrobial efficacy studies:

The zone of inhibition of standard drug solution, marketed formulation and prepared formulations (test) was found to be almost similar (**Fig. 15**). The present study results indicate that Moxifloxacin Hydrochloride retained its antimicrobial efficacy when formulated as an *in situ* gelling system.

FIG. 15: ANTIMICROBIAL EFFICACY OF MOXIFLOXACIN HYDROCHLORIDE IN GEL FORMING SOLUTION

Stability studies:

There were no signs of drug degradation and the drug was present uniformly distributed throughout the storage period as indicated in **Tables 8** and **9**. As observed from the long-term stability data and accelerated stability data, no attributes showed any significant change over the study period, it is apparent that the drug product will remain well within the acceptance criteria during the proposed shelf life.

TABLE 8: STABILITY DATA OF FORMULATION M6 AT 25ºC ± 2ºC/60% RH± 5% RH

Parameter		Initial	1 Month	2 Month	3 Month
Appearance		Clear	Clear	Clear	Clear
pH		6.75	6.71	6.70	6.66
Gelling ability		+++	+++	+++	+++
Viscosity in cps at 5 RPM	Before gelling	172.3	168.9	165.5	160.6
	After gelling	379.6	365.9	372.8	364.2
Drug Content		100.23±0.24	100.35±0.48	99.13±0.36	98.76±0.75

TABLE 9: STABILITY DATA OF FORMULATION M6 AT 40ºC ± 2ºC / 75 ± 5 % RH

Parameter		Initial	1 Month	2 Month	3 Month
Appearance		Clear	Clear	Clear	Clear
pH		6.76	6.65	6.59	6.52
Gelling ability		+++	+++	+++	+++
Viscosity in cps at 5 RPM	Before gelling	172.3	166.3	160.3	158.9
	After gelling	379.6	371.3	362.5	360.8
Drug Content		100.23±0.24	99.01±0.74	97.90±0.61	97.16±0.49

Temperature cycling / Freeze thaw studies:

Thermal cycling studies or freeze-thaw cycling studies are recommended to ascertain the effect of extreme temperature fluctuations during shipping through various climatic zones, seasonal fluctuation in temperature and mode of transport on the physical stability of the drug products. These studies are generally desirable for those drug products which may undergo phase separation, loss

of viscosity, precipitation, and change in particle size distribution.

Formulations were subjected to the freeze-thaw cycle test. Three freeze-thaw cycles were carried out wherein each cycle includes formulations stored at -20°C to -10°C for 24 hours and +40°C for 24 hours. The formulations were evaluated for visual appearance, pH, drug content, gelling ability and viscosity.

DISCUSSION: The developed formulations for Moxifloxacin Hydrochloridewere prepared using combinations of polymers viz.Sodium alginate (Keltone LVCR) and HPMC**.** Gelation studies revealed that, the *in situ* gelling systems gelled instantaneously when in contact with STF. The formed gels would enhance ocular contact time of the drug in eye. From rheological studies, it was concluded that, formulations exhibited pseudoplastic rheology and the viscosity was directly dependant on the polymeric content of the formulations [21, 22]. These results suggest that alginate changed to the gel phase upon exposure to lachrymal fluid. Addition of HPMC (E50LV, E15LV and K4M) as the viscosity-enhancing agent showed synergistic effect and a decrease in the amount of alginate in the preparation could be achieved.

Ophthalmic formulations should have a pH range of 5 to 7.4 to avoid the discomfort after instillation. pH of all formulations was found to be satisfactory. Thus there would be no irritation to the patient upon administration of the formulation. The *in vitro* diffusion data indicated that the formulations M6 and M12 showed better diffusion profiles and sustained effect when compared with other formulations. All the formulations showed an initial burst release. Prolonged release in the later stage can be attributed to the slow diffusion of the drug through polymer matrix.

The initial burst release of the drug can be explained by the fact that, the *in situ* gelling system is formulated in water and hence the polymer was completely hydrated. When it comes in contact with STF and gelation occurs, a prehydrated matrix is formed in which hydration and water penetration no longer limit drug release leading to an apparent diffusion-controlled release. The *in vitro* and *ex vivo* diffusion studies showed prolonged drug release over 12 hours period as compared to marketed eye drop.

Bioadhesion test is used to ensure that the formulation after forming gel inside the eye cavity remains adhered to the mucous membrane of the eye. HPMC contains an abundance of hydroxyl and ether groups along its length, which is responsible for the mucoadhesive properties [23]. It can also be substantiated due to the presence of Sodium Alginate a polymer with high glucuronic acid content which forms 3 dimensional ionotropic hydrogel matrices, generally by preferential interaction of G moieties resulting in formation of homogenous gels and also due to the low surface tension (31.5 mN/m) of the alginate, which is lower than the critical surface tension of the mucin coated cornea (38 mN/m), resulting in good spreading and adhesion [24].

CONCLUSION: Drug delivery to ocular mucosa for the treatment of ocular disease is associated with many obstacles. After instillation of conventional eye drops major fraction of the instilled dose is lost while less than 5% of the applied drug penetrates the cornea and reaches intraocular tissues. Ocular efficiency of topically applied drugs is influenced by the corneal contact time, most common method of improving ocular availability of drugs is to increase precorneal residence time by using *in situ* gels. The developed In *situ* gel forming solution showed better physico chemical attributes than the conventional ophthalmic formulations. Hence, the developed *in situ* gel forming solution can be used as an alternative to conventional eye drops for the treatment of various ocular infections by increasing the precorneal residence time and thus reducing the dosage interval.

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