Thermosensitive In situ Gel for Ocular Delivery of Lomefloxacin

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ABSTRACT

The temperature sensitive in situ gel formulations, undergo phase transition from liquid to semisolid gel upon exposure to physiological eye temperature. These are free-flowing liquid at room temperature and easy to administer into the eye as drops. They undergo in situ phase transition to form a strong gel that is capable of withstanding shear forces in the culde-sac and of sustaining drug release at physiological conditions. Lomefloxacin hydrochloride is a broad spectrum, second generation fluoroquinolone antibiotic used to treat various conjunctival infections. Present work describes the formulation of thermoreversible sol gel of Lomefloxacin hydrochloride using Pluronic F127, Pluronic F68 and sodium alginate. The prepared formulations were evaluated for various parameters such as clarity, pH, drug content, gelation capacity, gelation temperature, gelling strength, viscosity, in vitro release studies, pharmacokinetics studies, ex vivo permeation studies, antimicrobial activity animal studies and short term stability studies. The pH was in range of 7-7.5. The viscosity of the formulations at 25\(^\circ\)C and 37\(^\circ\)C was in the range of 50-60 cps and 1590-3370 cps respectively. The results obtained were in agreement with the phase transition and there was no significant change in the viscosity of formulation when diluted with simulated tear fluid. The in vitro drug release revealed a sustained profile of 8 hours and optimized formulation showing 89.3% of release. Short term stability study indicated that 4\(^\circ\)C is appropriate storage condition for the formulations.

Key words: Lomefloxacin, Pluronic F127, Thermoreversible sol gel, Cold technique, Phase transition, Shear thinning systems.

INTRODUCTION

Lomefloxacin is a difluoroquinolone antibacterial agent having wide spectrum of antibacterial activity, against gram-positive and gram-negative organism.\(^1\) Lomefloxacin when given in eye drops forms there is drug loss due tear drainage and nasolacrimal drainage which leads to shorter residence time of the drug which in turn reduces the corneal contact time ultimately leading to poor bioavailability and frequent dosing of the dosage form.

Drugs are commonly applied to the eye for a localized action on the surface or in the interior of the eye.\(^2\) The major problems in conventional liquid ophthalmic formulations are washing out of drug from the pre-corneal area immediately upon instillation so to increase pre-corneal residence time and ocular bioavailability, different ophthalmic delivery system such as viscous solutions, ointments, gels, suspensions or polymeric inserts are used. But because of blurred vision (e.g. ointments) or lack of patient compliance (e.g inserts), these formulations have not been widely accepted. These problems can be overcome by development of different delivery system such as In situ gel which is administered as liquid & undergoes a phase transition to semisolid gel upon exposure to physiological environment.\(^4\)

The word in-situ is derived from Latin which means “in its original place or in position”. A greater attention has been focused on the polymeric in situ gel because of its advantages over conventional ophthalmic formulations such as ease administration, reduced frequency of administration, improved patient compliance and they do not require...
organics or copolymerization agents because of which they have gained increasing attention.\textsuperscript{4,5} Pluronic 127 is a block copolymer that consists of polyethylene oxide (PEO) and polypropylene oxide (PPO) units, is known for exhibiting the phenomenon of reverse thermal gelation under a certain concentration and temperature. At a concentration of 18\% (w/w) or higher in aqueous solution, pluronic 127, is transformed from a low viscosity solution to a gel under the ambient temperature. But this lower concentration solution will lose its gelation ability after diluted by lacrimal fluid. Therefore, pluronic 68 (P188), was added to pluronic 127 solution as a regulatory substance and exhibited a good perspective to increase the gelling temperature (GT) of Pluronic 127. Different gel enhancing polymers has been used in combination with pluronic such as sodium alginate.\textsuperscript{6}

Thus aim of present work was to formulate Thermo sensitive sol-gel system for ocular delivery of lomefloxacin in order to increase the residence time of drug, sustained drug release and reducing dosing frequency of formulation by varying the concentration of Pluronic 127, pluronic 68 and sodium alginate.

**EVALUATION OF AN IN SITU GEL**

**Clarity & pH**

Clarity is one of most essential parameter for opthalmic preparations. All prepared formulations were evaluated for clarity by visual observation against black and white background and pH was determined using digital pH meter.\textsuperscript{8}

**Drug content**

Prepared \textit{in situ} gel formulation (equivalent to 10 mg of pure drug) was taken and diluted to 100 ml with freshly prepared Simulated tear fluid (Composition: Nacl-0.67 g, NaHCO$_3$-0.20, CaCl$_2$, 2H$_2$O-0.008 g and distilled water to 100 ml) (STF). From this 1 ml was withdrawn and diluted to 10 ml using STF. The absorbance was measured at 280 nm against STF as blank by using UV-Visual spectrophotometer.\textsuperscript{8}

**Gel strength**

Prepared formulation 25 g was placed in 100 ml graduated cylinder and gelled at 37\°C using thermostat. A 14 g weight is placed on to gelled form and allowed to penetrate 5 cm. Time required for penetration is noted and reported as the gel strength.\textsuperscript{9}

**Gelling capacity**

The gelling capacity of the \textit{in situ} gel formulations was determined by placing a drop of formulation in a test tube containing 2 ml of simulated tear fluid freshly prepared and equilibrated at 37\°C ± 2\°C and visually assessing the formation of gel, noting the time for gelation and the time taken for the formed gel to dissolve.\textsuperscript{10}

**Measurement of gelation temperature (GT)**

Sample solution of 10 ml was taken in a transparent beaker and placed in a water bath. The whole assembly was placed on magnetic stirrer and gradually temperature of the water bath was increased with continuous stirring at minimum rpm. The temperature at which the magnetic bar stopped moving due to gelation is noted as the gelation temperature.\textsuperscript{7}

**Effect of dilution on Gelling Temperature**

The measurements were made at 15-37\°C, in order to mimic the temperature in the conjunctival sac of the eye. The GT was defined as the point where a sudden shift in shearing stress was observed. In order to imitate the condition of eye the formulations were mixed with simulated tear fluid in a ratio of 40:7 as the normal tear volume present in eye is 7 µl.\textsuperscript{7}

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**Materials & Methods**

**Materials**

Lomefloxacin Hydrochloride was obtained as a gift sample from IPCA pharmaceuticals Ltd. Gurgaon (Mumbai). Pluronic 127 and Sodium alginate was purchased from Hi Media pvt ltd (Mumbai). Pluronic F68 was procured from Ozone International Mumbai. All chemicals used were of analytical grade.

**Method of preparation**

All the three polymers that is Pluronic 127, Pluronic 68 & sodium alginate were mixed in appropriate quantity of distilled water and stirred separately for 1 h on magnetic stirrer and refrigerated overnight at 4\°C. Next day, Pluronic 127 & Pluronic 68 polymer solutions were mixed together with continuous stirring for 1 h and again kept overnight at 4\°C. Sodium alginate solution along with benzalkonium chloride were added to above mixture and mixed with continuous stirring for 1 h. This mixture was added to specified drug and sodium chloride solution in distilled water with continuous stirring until it dissolves and further it was adjusted to pH 7.4 by adding 0.1 N sodium hydroxide solution. The formed \textit{in situ} gel was stored in the refrigerator.\textsuperscript{7} Nine formulations were prepared by varying the concentration of Pluronic 68 and sodium alginate as mentioned in (Table 1).
Rheological studies

Viscosity of the formulation was determined before and after gelation by using Brookfield’s rheometer (DV II+USA) using spindle number 21. Formulations were taken in a small volume sample tube and viscosity was measured at 5, 10, 20, 30, 40 and 50 rpm. Thermo stated water jacket was used in order to measure viscosity at 25°C ± 2°C and at 37 ± 2°C. The viscosity was measured before dilution with STF at 25°C ± 2°C and before and after dilution with STF at 37 ± 2°C.11

In vitro drug release of In situ gel system

In vitro release studies of the formulations were studied through a fabricated apparatus consisting of 1 ml of formulation placed in donor compartment using cellophane membrane (molecular weight cutoff 12,000–14,000 Dalton) which was soaked for 24 h in STF. The donor compartment was immersed in the receptor compartment containing 50 ml of STF maintained at 37°C ± 1°C with constant stirring using magnetic stirrer. After regular time intervals, 1 ml was withdrawn from
receiver compartment and replaced with fresh STF. All samples were withdrawn in triplicates. Samples were analyzed for amount of drug released with UV-spectrophotometer at 280 nm against STF pH 7.4 as blank. In vitro release studies were carried out with the pure drug solution in order to compare its release profile with the prepared in situ gelling system of Lomefloxacin hydrochloride. To analyse the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted in to Zero order, First order, Higuchi matrix and Korsemeyer-Peppas model. By comparing the R² values obtained, the best fit model was selected.

Ex vivo corneal permeation studies

Goat corneas were used to study the permeation across the corneal membrane. Whole eyeball of goat were procured from a slaughter house and transported to laboratory in cold condition. They were maintained in normal saline at 4°C. The cornea was then carefully removed along with a 5-6 mm of surrounding scleral tissue and washed with cold saline. The study was carried out using Franz diffusion cell in such a way that corneal side remains continuously in contact with formulation in the donor compartment. 1 ml of the optimized formulation (LF5) was taken and mounted on goat cornea. The receptor compartment was filled with STF pH 7.4 at 37°± 1°C. The receptor medium was stirred on the magnetic stirrer. 1 ml of samples was withdrawn at different time interval and absorbance was taken by UV spectrophotometer. Receptor compartment was replenished with an equal volume of STF (pH 7.4) at each time interval. The percent drug release was plotted against time to get dissolution curves.

Antimicrobial studies

The microbiological studies were carried out to ascertain the biological activity of best sol-to-gel system. This was determined by using Gradient Diffusion Method (Dig well technique). Sterile solution of pure drug was used as Standard. Standard solution and the developed formulation (optimized test solution LF5) were taken in separate cups bored into sterile Nutrient agar previously seeded with Staphylococcus aureus organism. After allowing diffusion of solution for two hours, the plates were incubated for 24 h at 37°C. The zone of inhibition was compared with that of the standard.

Isotonicity studies

Isotonicity has to be maintained to check tissue damage. Solutions of Sodium Chloride of three different concentrations were prepared to obtain hypertonic (3% w/v), hypotonic (0.2% w/v) and isotonic (0.9% w/v) concentrations. Four clean slides were taken. They were labeled as hypertonic (HT), hypotonic (HP), isotonic (IS) and test (T). A small drop of blood was applied to the center of each slide along with a drop of heparin solution (1%w/v) to prevent coagulation of blood. A drop of each test solution was placed on the respective slides. A drop of optimized formulation (LF5) was placed on the slide labeled as test (T). Using the edge of the cover slip, the contents were mixed and observed under microscope at 45X magnification to observe the morphology of RBCs.

Ocular irritancy test

Ocular irritation studies were performed on male albino rabbits weighing 1-2 kg. The modified Draize technique was designed for the ocular irritation potential of the ophthalmic product. Approval of the Institutional Animal Ethic Committee (resolution No. KLECOP/IAEC/Res.20-09/08/2014) was taken prior to the commencement of the study. They were divided in group of 3 in which each group was having 6 Rabbits. 2 drops of solution was placed in the lower cul de sac of rabbit eye once a day for a period of 7 days and irritancy was tested at the time interval of 1 h, 24 h, 48 h, 72 h and 1 week after administration. The rabbits were observed periodically for redness, swelling and watering of the eye.

Efficacy against bacterial conjunctivitis

Bacterial conjunctivitis was induced in rabbit eye by instilling bacterial strains of staphylococcus aureus culture. These were done by placing 2 drops of culture in cul de sac of rabbit eye. Treatment was initiated 48 h later. 18 rabbits were selected and divided into 3 groups that is Test, standard and control. 6 rabbits were kept in each group. Infection was induced in standard and test group rabbits while not induced in Control group. Test group were treated with optimized LF5 formulation and standard group with pure drug solution.

Dose of 2 drops were instilled in the cul de sac of rabbit eye once a day and animals were observed for redness, mucoidal discharge and swelling of eyelids.

Short term stability studies

Stability testing of pharmaceutical products is done to ensure the efficacy, safety and quality of active drug substance in dosage forms during the storage. Optimized formulation LF5 was subjected to stability studies at 4°C and at room temperature that is 25°C for a period of 3 months. The samples were withdrawn after 30, 60 and 90 days and evaluated for parameters such as pH, Drug
content, gelation temperature, \textit{in vitro} drug release and viscosity before and after gelation at 5 rpm.\textsuperscript{19}

**RESULTS & DISCUSSION**

**Clarity, pH and visual appearance**

All the prepared formulations were free flowing liquid at room temperature, clear, and free of any particulate matter. Normal physiological pH of eye ranges from 7 to 7.4. The pH of all formulation was found in the range of 7.0 to 7.5 (Table 2) which was within the physiological range of eye hence would not cause any irritation upon administration.

**Determination of drug content**

Drug content was determined by UV spectrophotometer at 280 nm using simulated tear fluid as the dissolution medium. Drug content of formulations LF1-LF9 were in the range of 95.55 to 98.18% (Table 2) indicating uniform distribution of drug.

**Gel strength**

Gel strength is determined at physiological temperature of eye (37°C ± 1°C). Gel strength observed for the \textit{in situ} gel formulations is as shown in (Table 3). There was gradual increase in the gel strength of formulations with increase in concentration of Pluronic F68 due to enhancement in ethylene oxide/propylene oxide ratio of pluronic 127 when combined with it.\textsuperscript{20} Formulations containing 1.5% of sodium alginate showed higher strength as compared to 0.5% concentration. This may be due to interaction of sodium alginate with pluronic leading to increase in the molecular weight of hydrophobic molecules.

**Gelling Capacity**

The gelling capacity of all Temperature Sensitive formulations is as shown in (Table 3). All formulations showed immediate gelation and remained for extended period of time. The formulations should have an optimum gelling capacity, so that after instillation into the cul de sac of eye as a liquid (drops), it would undergo a rapid sol-to-gel transition and would preserve its integrity without dissolving or eroding for a prolonged period of time.\textsuperscript{8}

**Gelation Temperature**

Gelation temperature of the formulation is in the range of 31.1°C to 39.3°C as depicted in Figure 1. It was observed that with increment in the concentration of pluronic F68, the gelation temperature increases but up to a certain limit. Pluronic F127 alone showed gelation temperature at 30-32°C which was increase to physiological temperature by addition of pluronic F-68. Pluronic F-68 with 1% concentration showed gelation temperature in the range of 35.2°C-31.1°C for formulations LF1-LF3. It showed gelation temperature from 37.2-34.2 for formulation LF4-LF6 with 2% concentration and 39.3-37.6 for LF7-LF9 with 3% concentration. There was gradual decrease in the gelling temperature even though concentration of Pluronic F68 remains same for the respective three formulations. This decrease may be due to bioadhesive polymer that is sodium alginate. With gradual increase in the concentration of sodium alginate from 0.5 to 1.5 there was decrease in gelation temperature. The gelation lowering effect of bioadhesive polymer could be explained by their ability to bind to the polyoxyethylene chains present in the pluronic molecules. This will promote dehydration, causing an increase in entanglement of adjacent molecules and extensively increasing intermolecular hydrogen bonding which will lead to gelation at lower temperature.\textsuperscript{21} Ideally \textit{in situ} gel which are free flowing liquid at room temperature turns to gel at physiological temperature are considered optimized as gelation at lower or higher physiological temperature will either cause difficulty in administration or get drained by tear fluid respectively. Thus by variation in the concentration of polymer the gelling temperature can be attained in the range of physiological temperature of eye. LF5 formulation contain 2% pluronic 68 and 1% sodium alginate showing gelation at temperature 37°C (physiological temperature of eye). Thus it is optimized.

**Effect of Dilution on Gelling Temperature**

The phase transition temperature for all formulations was as tabulated in (Figure 1). No significant change was observed in gelation temperature when diluted with STF showing the efficacy of formulation to remain in gel form even when diluted with tear fluid.

**Rheological studies**

An ophthalmic formulation must have an optimum viscosity that will allow easy instillation into the eye as liquid drops and which would undergo rapid sol-to-gel transition upon instillation in the eye. Viscosity of all formulation is depicted in Figures 2, 3 & 4. It was observed that with increase in temperature there was drastic increase in viscosity. This may be attributed to the miceller formation of PEO/PPO ratio of Poloxomer at higher temperature. All the formulation exhibited Pseudo plastic behavior that is with increase in shear rate they showed decrease in viscosity. This shear thinning behavior is responsible for uniform distribution of drug on the corneal surface of eye. The high viscosity at lower shear rate helps to increase the contact time of \textit{In situ} gel on the corneal surface.
This pseudo plastic behavior of gel that is with increase in shear rate decrease in viscosity is mainly necessary as range of shear rate experienced during relative movement of eyelids and globe is extremely wide which leads to ocular shear rate of about 0.03 s$^{-1}$ during interblinking periods and 4250-28500 s$^{-1}$ during blinking. Also it was found that there was no significant effect of tear fluid on the viscosity of gel confirming efficacy of gel to remain stable in cul de sac of eye.

**In vitro drug release**

The release of drug from these gels was characterized by an initial phase of high release that is burst effect. However, as gelation proceed the remaining drug was release at a slower rate. (Second phase of moderate release). This biphasic pattern of release is a characteristic feature of matrix diffusion kinetics. The results showed that the formed gels had the ability to retain the drug for the period of 8 h (duration of study). It was found that the drug release of the formulations were in the range of 76.99-90.48%. The release rate showed that with increase in concentration of PF-68 and Sodium alginate there was decrease in the release rate of the drug which functioned as an increasingly resistance behavior to drug release. Due to increase in the number and size of the micelle within the gel structure it leads to enhanced resistance resulting in formation of more dense gel giving sustained release. It was also noted that with increment in sodium alginate concentration there was decrease in the release rate of drug proving interaction of sodium alginate with the micellar entanglement giving more dense arrangement to gel. Above results indicated that the structure of gel functioned as a barrier to drug release. Such enhanced resistance may be due to increase in size of micelle within the gel structure which leads to higher viscosity and sustained drug release. The *in vitro* release profile of formulations are shown in (Figure 5). The release data were fitted to various kinetics models in order to find out mechanism of drug release. All the formulation in the study were best expressed by Higuchi indicating the release process was diffusion controlled. The diffusion exponent (n) values for all formulations were less than 0.5 indicating fickian mechanism of drug release. The *in vitro* release profile of LF5 was compared with pure drug solution. The cumulative percentage drug release after first 15 min was found to be 35.36% and 20.47% (Figure 6) for the pure drug and optimized LF5 formulation respectively. At the end of 3 h the cumulative percentage drug release was 98.39% and 41.13% for pure drug solution and LF5 formulation.

**Ex vivo corneal permeation studies**

The permeation of the optimized formulation LF5 through goat cornea was 79.28% (Figure 7). The drug diffuse through the corneal membrane was less as compared to drug diffuse with dialysis membrane. These may be because the cornea is made up of epithelium (lipophilic), stroma (hydrophilic) and endothelium (less lipophilic than epithelium) which act as lipophilic–hydrophilic barrier for corneal penetration while dialysis membrane act as a mechanical barrier.

**Antimicrobial activity**

The result of the antimicrobial efficacy tests shown in (Figure 8). The study indicates that Lomefloxacin hydrochloride retained its antimicrobial activity after formulated into an *in situ* gel. The drug was active against the selected staphylococcus aureus organism as indicated by zone of inhibition.

**Isotonicity studies**

The optimized test solution did not showed swelling or shrinkage of blood cells.

**Ocular irritation studies**

The Optimized formulation LF5 was non irritating with no ocular damage. There was no signs of redness, mucous formation or inflammation.

**Efficacy against bacterial conjuctivities**

During the treatment eyes were observed for the redness, lacrimal secretion, mucoidal discharge and swelling of eyelid. The improvement in the symptoms was observed with sol to gel systems. Symptoms associated with conjunctivitis were reduced faster with sol-gel formulation as compared to pure drug formulation. Sol to gel system took 5 days to cure the infection while pure drug solution was unable to cure in 5 days.
Figure 2: Viscosity of formulation F1-F3 at 25°C ± 1°C & 37°C ± 1°C

Figure 3: Viscosity of formulation F4-F6 at 25°C ± 1°C & 37°C ± 1°C

Figure 4: Viscosity of formulation F7-F9 at 25°C ± 1°C & 37°C ± 1°C

Figure 5: Comparative diffusion profile of formulations LF1-LF9

Figure 6: Comparative diffusion profile of formulations LF5, and pure drug solution
Short term stability studies

The physical appearance of the formulation LF5 stored at room temperature changed slightly. The viscosity was found to increase when stored at room temperature but not in significant amount. The drug content of the formulation stored at 4°C showed no significant variations but of that stored at room temperature varied significantly. This variation in the drug content can be attributed to the dehydration of gel formulation. From the results it can be interpreted that the prepared sol gel LF5 can be best stored at 4°C ± 1°C.

CONCLUSION

Thermoreversible sol gels are shear thinning systems which show temperature dependent gelation. Lomefloxacin Hydrochloride is a broad spectrum antibiotic effective against gram positive and gram negative Bacteria. A combination of Pluronic F127 and Pluronic F68 along with sodium alginate was used to prepare the gel base.

The FTIR studies revealed the presence of compatibility between the drug and the formulation excipients. PF127 (18%w/v), PF68 (2%w/v) and Sodium alginate (1%) in combination were selected as the optimized polymer concentration.

The rheological studies confirmed sol to gel transition at physiological eye temperature.

The pH of all formulations was in between 7 to 7.4.

The drug content for all formulations was in between 95.55 to 98.18 % which ensures dose uniformity in the formulation.

The gelation studies showed that, prepared insitu gels instantaneously when contacted with STF. The formed gels would enhance the ocular contact time of Lomefloxacin Hcl in eye which in turn prolong the residence time of drug.

From the Rheological studies it was observed that all formulation exhibited pseudoplastic rheology as evidence by decrease in viscosity with increasing in angular velocity.

In-vitro drug release study indicated controlled drug release over a period of 8 hours.

Formulation LF5 containing 18% w/v PF 127, 2 %w/v PF68 and 1 % sodium alginate was selected as the optimized one as it showed gelation near physiological temperature and 89.38% drug release at the end of 8 hours. All the formulations in this study were best expressed by Higuchi’s model as the plots showed good linearity. (R2: 0.973-0.981) The linearity of the plot indicated that the release process was diffusion controlled.In peppas model n values for formulation ranged from 0.338-0.407 indicating that the release mechanism was Fickian release (n< 0.5).

Formulation LF5 was selected as the best formulation because it exhibited good gelling capacity, optimum viscosity and 89.38% in vitro drug release in 8 hours study. The optimized formulation LF5 showed satisfactory tolerance during rabbit eye irritation studies. Formulation LF5 cured infection of bacterial conjunctivitis within 5 days.

The results of short term stability studies indicated that, the most suitable storage condition for Insitu gel of Lomefloxacin Hcl was 4°C ± 1°C.

The optimized formulation exhibited promising antimicrobial activity against the clinical isolates of staphylococcus aureus.

The formulation can be used as once a day application in the treatment of Bacterial conjunctivitis. The future prospects include improving the water solubility of drug, In vivo Pharmacokinetic and Pharmacodynamic studies, Long term stability studies And IVIV Correlation studies.
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CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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PICTORIAL ABSTRACT

SUMMARY

- In the present study thermoreversible sol gel of Lomefloxacin hydrochloride, a second generation antibiotic, with the combination of Pluronic F127, Pluronic F68 and sodium alginate was developed.
- The FTIR studies revealed the presence of compatibility between the drug and the polymers.
- The method of preparation chosen was “cold technique” as described by Schmolka. The prepared formulations were evaluated for various parameters such as clarity, pH, drug content, gelation capacity, gelation temperature, gelling strength, viscosity, invitro release studies, pharmacokinetics studies, exvivo permeation studies, antimicrobial activity animal studies and short term stability studies.
ABBREVIATIONS USED


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