Development and Characterization of Occular Delivery of Moxifloxacin In-Situ Gel

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A B S T R A C T

In-situ gelling systems are viscous polymer-based liquids that exhibit rapid sol-to-gel transformation triggered by external stimulus such as temperature, pH etc. on instillation to the ocular surface. The aim of the present study was to formulate and evaluate pH responsive in-situ gel of Moxifloxacin, in view of increasing precorneal residence time and bioavailability of the drug for ocular delivery by the use of the gelling agent like sodium alginate (ion sensitive) and viscosity enhancers like HPMC. Moxifloxacin is a broad spectrum 8-methoxyfluoroquinolone antibiotic used for the treatment of bacterial conjunctivitis, keratitis and surgical prophylaxis and well tolerated with minimal ocular side effects. However, rapid dilution on instillation, wash out, poor retention of drug concentration delimit the therapeutic benefits of the drug when used in form of conventional eye drops. The developed formulations were evaluated for clarity, pH measurement, drug content, rheological study, isotonicity, ocular irritancy study, sterility study, antimicrobial activity and in-vitro permeation study. Thus, in-situ gel based systems containing gums can be a valuable approach for ocular delivery when compared to conventional systems.

Keywords: Ophthalmic delivery, Hydrogels, pH induced method, Gelling capacity.

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1. Introduction

Conventional Ophthalmic delivery systems like eye drops result in poor ocular bioavailability due to ocular anatomical and physiological constraints, which include the relative impermeability of the corneal membrane, tear and nasolacrimal drainage. Most of the topically applied drugs are washed off from the eye by various mechanisms includes lacrimation, tear dilution and the residence time of most conventional ocular solutions ranges between 5-25 min. Only 1-10% members of topically applied drugs is absorbed. So, these may be overcome by fabricating the drug as a formulation that undergoes instantaneous in-situ gel formation upon Ophthalmic administration. These in-situ gelling systems consists of polymers that exhibit rapid sol-to-gel transition after instillation due to change in specific physicochemical parameters (pH, temperature, ionic strength) occurring in the eye. [1-2]

The polymers chosen to prepare Ophthalmic hydrogels should meet some specific rheological characteristics.[3-4] It is generally well accepted that the instillation of a formulation should influence tear behavior as little as possible. Because tears give pseudo-plastic behaviour, pseudo-plastic vehicles would be more suitable as compared to Newtonian formulations, which have a constant viscosity independent of the shear rate, whereas pseudo plastic solution exhibit decreased viscosity with increasing shear rate, there by offering lowered viscosity during blinking and stability of the tear film during fixation.[5] Many of Ophthalmic in-situ forming gels investigated to formulate with carboxomers or cellulose derivatives. Various preparations like Ocular inserts, hydrogels etc., have been developed to prolong the contact time on Ocular surface, but the main problem with these preparations is patient compliance and blurred vision so that the liquid dosage forms are more preferable.

Sodium alginate, an Ophthalmic gel forming mucoadhesive polymer which undergoes instantaneous gel formation due to formation of calcium alginate by virtue of its interaction with divalent cation (Ca+2) present in lacrimal fluid (pH 7.4). Hydroxy Propyl Methyl Cellulose (HPMC K4M and E5 0LV) was further incorporated as a viscosity enhancer in order to achieve the desired consistency so as to facilitate sustained drug release.

2. Materials and Methods

Moxifloxacin was obtained from A-Z Chemical Pvt. Ltd., Chennai, HPMC, HPMC K15, Sodium Alginate from S.D Fine chemicals, India. All other chemicals and reagents were of analytical grade satisfying Pharmacopoeial standards.

**Calibration curve of Moxifloxacin at 293nm**

100 mg of Moxifloxacin was dissolved in 100 ml distilled water to get a concentration of 1mg/ml. From this, 10 ml was taken and made upto 100 ml with distilled water(100 mcg/ml). From this 100 mcg/ml solution, a series of concentrations 2,4,6,8,10 mcg/ml were prepared and absorbance of these solutions was measured against a blank of distilled water at 293 nm using UV-Visible International Journal of Current Trends in Pharmaceutical Research spectrophotometer. Calibration curve was plotted against concentration versus absorbance as given figure 1.

**Preformation Studies**

**Physical Observation**

The drug and excipients were packed in closed vials and kept in accelerated atmospheric conditions for a period of four weeks to check the morphological characteristics such as colour, odour.

**Drug-polymer interaction studies by FTIR, DSC**

The presence of incompatibility between drug and excipients were evaluated by performing drug-polymer interaction studies using Infrared spectroscopy and Differential Scanning Colorimetry.

**FTIR**

The pure drug, polymer and drug-polymer blends were subjected to FTIR studies by potassium bromide pellet method. The samples were thoroughly mixed with dry powdered potassium bromide (KBr). The scanning range was 4000-400 cm⁻¹ and resolution was 1 cm⁻¹. The mixture was compressed into disc using dies. Disc was placed in spectrophotometer and spectrum was recorded.

**DSC**

Differential Scanning Colorimeter (DSC) is a thermo analytical method used to study the drug and polymer interaction in which both test and reference were heated from 20-450 °C and same criteria of temperature maintained during observation. The drug and polymer were scanned individually. DSC is majorly used for checking the melting points and glass transition temperatures for drug, polymers are available and the experiment can show promising polymer degradation by the lowering of the projected melting point to study the interaction between drug and polymers. The DSC thermo grams of pure drug, polymer and the composition of drug–polymers were recorded in DSC analyzer model Universal V4.5A at a heating rate of 20°C/min from 0 to 450°C in nitrogen atmosphere.

**Formulation of Moxifloxacin in-situ gel**

Moxifloxacin in-situ gels of different formulations were formulated by employing "pH induced method" with various ratios of sodium alginate, HPMC, HPMC K15, Sodium alginate, HPMC, HPMC K15 of required quantity were heated slightly for 15 min, then cooled with Stirring (Table 1). After cooling, sodium chloride, methyl paraben and drug solution were added to the polymer solution with continuous stirring and volume was made up to 100 ml and this solution was filtered through 0.2 mm filter paper.[6]

**Evaluation**

**Clarity:** The clarity of the prepared formulation was observed by visual inspection under a good light, viewed against a black and white back ground, with the contents set in motion with a swirling action. Also it was observed for formation of turbidity or any unwanted particles dispersed in the solution.

**Measurement of pH**

By taking each formulation in a beaker, pH was measured using pH meter which was previously calibrated using standard buffers of pH 4 and pH 7.
Drug Content
1ml of each formulation was dissolved in 100 ml pH 6.4 buffer, from which aliquots of 1ml was withdrawn and under suitable dilutions, drug content was determined using UV-Visible spectrophotometer.

Rheological Studies
The viscosity of the prepared formulations were measured by using Brookfield viscometer, by placing each prepared In-situ gel formulation in the sampler tube using spindle no. and keeping in the Brookfield viscometer

Isotonicity
Isotonicity is an important characteristic property of Ophthalmic preparation. All ophthalmic preparations were subjected to isotonicity testing, since they exhibited good gelling capacity & the velocity. Formulation mixed with few drops of blood & observed under microscope at 45x magnification & compared with standard marketed ophthalmic formulation.

Ocular irritancy studies
The optimized formulation was terminally sterilized and evaluated for in-vivo performance in animal model (Albino Rabbits). The protocol is approved by college ethical committee [930/PO/aa/2006/CPCSEA]. Two groups of rabbits [six in each group] were used for this study. They were housed and maintained in the animal house at room temperature. During the period study, they were fed standard diet and water. The animals were placed in cages and the eyes were marked as test and control (0.5ml), and the eyes were observed for the Ocular irritancy (includes the microscopic cornea, iris conjunctiva).

In-vitro diffusion studies
In-vitro diffusion studies were carried out by using bi-chambered donor, receiver compartment model (Franz diffusion cell). Accurately measured 1 ml of the formulation was spread uniformly on a dialysis membrane, which was in contact with receptor medium (pH 7.4 which will simulate the lacrimal fluid) which was placed on magnetic stirrer and temperature was adjusted to 37 ± 0.5°C with the speed of the rotation maintained at 50 rpm. The samples were withdrawn at periodic intervals and the amount of drug release was analyzed by using UV spectrophotometer at 293 nm against reference standard using simulated tear fluid as blank under suitable dilutions.

Sterility Studies
Preparation should be examined during usage. Sterile media was pipetted out by sterile pipette and then with sterile syringe aseptically transferred the specified volume of sample to fluid thioglycolate medium and Soyabean casein digest medium and incubated for 7 days at 30 to 35 °C for fluid thioglycolate medium and 20 to 25 °C for Soyabean casein digest medium and periodic observation were carried upto seven days to check the growth of microorganisms.

Antimicrobial activity
Antimicrobial studies were carried out to ascertain the biological activity of sol-to-gel systems against microorganisms. This was determined in the agar diffusion medium employing Cup plate technique. Sterile solution of marketed Moxifloxacin eye drops was used as a standard. The standard solution and the developed formulations (test solution) were taken into separate cups bored in sterile Agar previously seeded with organisms (Staphylococcus aureus and Pseudomonas aeruginosa). After allowing diffusion of solutions for two hours, the plates were incubated for 24 hr at 37°C. The zone of inhibition (ZOI) was compared with that of the standard. Each samples were tested in triplicate.

3. Results and discussion
The physical observation of Moxifloxacin and mixtures showed no change in their physical properties. This revealed that there was no significant reaction between the Moxifloxacin and mixtures. Drug-excipients compatibility study was performed by FTIR and DSC (Figure 2 to Figure 9). From the IR spectral study, it was observed that there was no significant change in the peaks of drug, polymer and drug-polymer mixtures and from the DSC, there was no change in the melting point. Hence, no specific interaction was observed between the drug and the polymers used in the formulations. Moxifloxacin in-situ gels of different formulations were formulated using “pH induced method” by using various ratios of sodium alginate, HPMC, HPMC K15 and further evaluated for different parameters like pH, appearance, drug content, in-vitro release studies, sterility test, Ocular irritation studies.

All the prepared formulations were very clear, without any turbidity, foreign particles and fibres. The pH of the formulations were determined by using a calibrated pH meter. pH of all the prepared formulations (Table No.2) was in between 6.2 to 6.4 which was found to be acceptable for Ocular delivery. The viscosity was directly dependent on the polymeric content of the formulations. Rheological studies revealed that the viscosity of formulation was in optimum range that will allow for easy instillation into the eye, which would undergo a rapid sol to gel transition. The drug content was determined to check the amount of the drug in each formulation. The drug content was found to be in acceptable range for all the formulations and it was observed F1 and F3 had shown high % drug content The viscosity of prepared formulation data’s were showed in table No.4.

In-vitro dissolution studies of in-situ gel (5mg) of Moxifloxacin showed in figure No.10 that percentage drug release was found to be in between 62.38-68.01% in 8 h. Thus the in-vitro dissolution test indicated the sustained
release nature of in-situ gel of Moxifloxacin. Also, it was observed the highest % drug release was for F3 formulation and it was 68.01%. Sterility studies revealed that there was no microbial growth in the formulation (Figure No.12) hence the formulations passed the sterility test. By antimicrobial assay it was identified that formulation has desired antimicrobial activity. It is estimated by zone of inhibition of microorganisms by the action of prepared formulation. After 7 days of examination, the cul-de-sac of the test rabbit does not show any redness and rashes so the formulation was found to be non-irritant.

Figure 1: Calibration curve of Moxifloxacin at 293 nm

Figure 2: FTIR spectra of Moxifloxacin

Figure 3: FTIR spectra of Polymer

Figure 4: FTIR spectra of Moxifloxacin & polymer

Figure 5: DSC curve of Moxifloxacin

Figure 6: DSC curve of HPMC

Figure 7: DSC curve of HPMC K15
4. Conclusion

Moxifloxacin was successfully formulated as in-situ gel using sodium alginate and HPMC, HPMC K15 for ocular delivery. Thus the above results indicated that the alginate and HPMC mixture can be used as an in-situ gelling vehicles to enhance ocular bioavailability and patient compliance. Physicochemical characterization and in-vitro drug release studies shown that the developed formulation (F3) may prove to be a viable alternative to conventional eye drops and ointment in terms of ease of administration with added benefits of sustained drug release which may ultimately result in improved patient compliance.

5. References


