JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Design and Development of pH-monitered *in situ* Gel of Lomefloxacin

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ABSTRACT:

Present Study was focused on Design and Development of in situ gel of Lomefloxacin HCL.Lomefloxacin HCL is an antibacterial agent which exhibits rapid precorneal elimination and poor ocular bioavailability, when given in the form of conventional ophthalmic solutions. To overcome this, an attempt has been made to formulate pH-triggered in situ gelling system of Lomefloxacin HCL to provide sustained release of drug based on polymeric carriers that undergo sol-to-gel transition upon change in pH. The Lomefloxacin HCL in situ gelling system formulated by using poly acrylic acid (Carbopol 940) and in combination with hydroxyl propyl methyl cellulose (HPMC) which acted as viscosity enhancing agent. The developed formulation was stable, non-irritant and provided sustained release over 8-hour period and it is a viable alternative to conventional eye drops.

KEYWORDS: In situ gel, Cabopol 940, pH, Lomefloxacin HCL

Article history:
Received 18 Dec 2012
Revised 14 Jan 2013
Accepted 25 Jan 2013
Available online 13 Feb 2013

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INTRODUCTION:

Ophthalmic delivery systems like eye drops result in poor ocular drug bioavailability due to ocular anatomical and physiological constraints, which include the relative impermeability of the corneal epithelial membrane, tear dynamics and nasolacrimal drainage. Most of the topically applied drugs are washed off from the eye by various mechanisms include lacrimation, tear dilution and the residence time of most conventional ocular solutions ranges between 5 and 25 minutes. Only 1-10% of topically applied drug is absorbed, and major part of drug absorbed systemically results in systemic side effects. [1-4]

The increase in the precorneal residence time of drug and consequently better bioavailability can be achieved by using delivery system based on the concept of *in situ* gel formation. These *in situ* gelling systems consist of polymer that exhibit sol-to-gel phase transitions due to change in specific physicochemical parameters (pH, Temperature, ionic strength) in the environment, cul-de-sac in the case of eye. The sol-to-gel phase transition on the eye surface depending on the different methods employed and they are: pH-triggered system (eg.Cellulose acetate hydrogen phthalate latex), temperature dependent system (eg.Pluronics and tetronics) and ion activated system (eg.Gelrite). Many of the ophthalmic *in situ* forming gels - Investigated to date have been formulated with carbomers (or) cellulose derivatives. The formulation and evaluation of an ophthalmic delivery system of an antibacterial agent, gatifloxacin based on *in situ* gelling system and showed improved rheological behaviour, enhanced ocular bioavailability and better patient compliance compared to conventional ophthalmic solutions. [6] pH-triggered in situ gelling system of puerarin and showed better pseudoplastic behavior of the fluid and *in vitro* release of gelling system were better than puerarin eye drops. Srividya, et al. studied ophthalmic delivery based on pH-triggered *in situ* gelling and showed *in vitro* release of more than 8-hour period.

The developed system was stable, non-irritant, and a viable alternative to conventional eye drops. Raida, et al. demonstrated the *in situ* gelling system of ciprofloxacin and showed controlled release of drug .They reported antimicrobial efficacy of selected formulations against bacterial strains and showed
prolonged antimicrobial effect. Jayanta Kumar Pandit, et al. investigated the *in situ* gelling system of indomethacin and showed the formulations were therapeutically efficacious and provided *in vitro* sustained release of drug over 8-hour period.[9]

The aim of the present study is to study the pH-triggered *in situ* gelling system of Lomefloxacin HCL, a s**econd** generation fluoroquinolone derivative used in the infection of the eye such as acute conjunctivitis.The recommended dosage of Lomefloxacin HCL for the treatment of bacterial conjunctivitis is 1or2 drops of 0.3% solution in the affected eyes for every 2 hours upto 8 times for 2 days, then 1 or 2 drops every 4 hours upto 4 times for next 5 days. A combination of carbopol and HPMC was investigated as a vehicle for the formulation of eye drops of Lomefloxacin HCL (0.3%) to form gel when instilled into the eye to provide sustained release of the drug to improve the patient compliance by reducing the frequency of administration.[10]

MATERIALS AND METHODS

Lomefloxacin HCL was gift sample from Ipca Limited, Ratlam, India. carbopol and hydroxyl propyl methyl cellulose (HPMC) Sodium Chloride and benzalkonium chloride S.D. Fine Chemicals Ltd., Mumbai. All chemicals used were of analytical grade.

Method of preparation:

The *in situ* gel formulation was prepared by changing the concentration and using different polymers. [11] Different concentrations of polymers were used to prepare ophthalmic solutions as per the composition shown in Table-1.The polymers were dissolved in Acetate buffer pH – 5 and allowed to hydrate. To this buffered polymeric solution Carbopol 940 was added. Lomefloxacin HCL was dissolved in sodium hydroxide solution (0.1N) separately and after adjusting the pH, benzalkonium chloride was added and filtered. The drug solution was then added to the polymeric solution under constant stirring until a uniform solution was obtained. Acetate buffer solution was then added to make up the final volume. The formulations were sterilized under the membrane filtration and then filled in vials under aseptic conditions, and further evaluations were carried out. [5]

Determination of visual appearance, clarity, pH and drug content:

The appearance and clarity were determined visually. The pH of the formulations was adjusted by using pH meter. The drug content of *in situ* gel was determined by taking sample (2ml) of *in-situ* gel in a 100ml volumetric flask and diluted with simulated tear fluid of pH 7.4 to get the concentration of 10g/ml (approximately).Then the absorbance was measured at max (281nm) using UV-spectrophotometer to calculate the percentage of drug content. [12]

Gelling capacity:

The prepared *in situ* gelling system was evaluated for gelling capacity in order to identify the composition suitable for use as *in situ* gelling system. The *in situ* gelling system was mixed with simulated tear fluid (in the proportion of 25:7 *i.e.* application volume 25µl and normal volume of tear fluid in the eye is 7µl) to find out the gelling capacity of the ophthalmic product. The gelation was then assessed visually by noting the time for the gelation and the time taken for dissolution of the formed gel .[13]

Rheological studies:

The relationship between contact time and the rheology was easily understood for viscosity enhanced ophthalmic solutions. It was noted from various literature that the formulations before gelling should have a viscosity of 5 to 1000 mpa and after gelling in the eye will have a viscosity from about 50-50,000 mpa. Rheological studies of the prepared formulations were carried out by Brookfield synchroelectric viscometer (LVDV Pro II) using spindle S18 (small sample adaptor).The viscosity of the formulations were determined at different speed conditions (10,20,50,75 to 100 rpm). [15]

***In vitro* release studies:**

The drug release from the prepared formulation was studied by placing the test solution in a circular Teflon cup of 2.5cm internal diameter and1.2cm depth. This was in turn placed on an inverted USP basket kept inside a 250-ml beaker containing 200 ml of simulated tear fluid (pH7.4) as a dissolution medium and it was stirred by a magnetic stirrer (50 rpm) maintained at a temperature of 37±1 ?C. Samples (1ml) were withdrawn at regular intervals and replaced with an equal volume of fresh medium. The absorbance of the diluted samples was measured at max (281nm) by UV-spectrophotometer using simulated tear fluid as a blank to calculate amount of drug release from in situ gel. The percentage of drug release was

plotted against time to find the drug release pattern of all *in situ* gel preparations [14]. Then, the release of selected *in situ* gelling system was compared with that of marketed eye drops.

Antimicrobial activity:

Antimicrobial efficiency 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 Lomefloxacin HCL eye drops was used as a standard. The standard solution and the developed formulations (test solution) were taken into separate cups bored into sterile Muller Hinton Agar (MHA) previously seeded with organisms (*Staphylococcus aureus, E. Coli and Pseudomonas aeruginosa*). After allowing diffusion of solutions for two hours, the plates were incubated for 24 h at 37 C. The zone of inhibition (ZOI) was compared with that of the standard. [16]

Sterility testing:

IP method (1996) was followed for the sterility testing of eye drops. Sterility testing was carried out by incubating

formulations for not less than 14 days at 30 to 35 C in the fluid thioglycolate medium to find the growth of bacteria and at 20 to 25 C in the soyabean-casein digest medium to find the growth of fungi in the formulations. [17]

Ocular irritancy studies:

Ocular irritation studies were performed on male albino rabbits weighing 1-2kg.The modified Draize technique was designed for the ocular irritation potential of the ophthalmic product. According to Draize test, the eye drops (100µl) was normally placed in the lower cul-de-sac and irritancy was tested at the time interval of 1hr, 24hrs, 48hrs, 72hrs, and 1 week after administration. The rabbits were observed periodically for redness, swelling and watering of the eye. [18]

Accelerated stability studies:

The ophthalmic formulations in amber colored vials were used for a short term accelerated stability studies by storing at 40 ±2 C and 75±5% RH as per modified ICH guidelines. Samples were periodically evaluated for appearance, pH, gelling capacity and drug content during the study period. [19]

FT-IR studies:

The possibility of drug-excipient interactions were investigated by FTIR studies. The FTIR graph of pure drug and combination of drug with excipient were recorded using KBR pellets. [20]

RESULTS AND DISCUSSION

Six formulations of Lomefloxacin HCL *in situ* gelling systems were prepared by using various concentrations of carbopol 940 along with different grades of hydroxyl propyl methyl cellulose in different ratio as per formula given in Table-1. All the formulations had fixed drug concentration of (0.3%w/v) Lomefloxacin HCL.

Appearance, clarity, pH and drug content:

The appearances of all formulations were light yellow in colour and were clear except the formulation F-2. The pH of all the formulations was found to be within the range of 5.0 to 5.4, which is desirable for the ophthalmic formulations. The drug content of all the formulations was within the range of 98.35% to100.13%, showed the uniform distribution of drug in the ophthalmic formulations and the results are shown in Table-2

Gelling capacity:

The viscosity and gelling capacity plays important role for in situ gelling system. The formulation should have an optimum viscosity for easy instillation into the eye as a liquid which undergo sol-to-gel transition. Formulations CH 11,15,21 and 25 prepared from Carbopol940(0.3 to 0.4%)/HPMC (E4M0.4 to 0.6), Carbopol 940(0.3%)/HPMC (E50LV) 1.5% showed better gelling capacity. The other formulations were not having desirable gelling capacity. The grading for gelling capacity was shown in Table-2.

Rheological studies:

The pseudo plastic character of precorneal tear film should be disturbed less by the administration of ophthalmic products. The ocular shear rate is about 0.03 s during inter-blinking periods and 4250 – 28500 s during blinking. So, the viscoelastic fluids having high viscosity under low shear rates and low viscosity under high shear rates which is called as pseudoplastic fluid is often preferred The viscosity of the formulations (CH-1 to CH-16) ranged from 3107 cps.The rheological study of the formulations exhibited decrease in viscosity on increase in shear rate because of the pseudoplastic behavior of the formulations Moreover, the pseudoplastic property of these formulations may be in favour of sustaining the release of drug in the conjunctival sac of the eye, and also without blinking difficulty for undergoing shear thinning.

***In vitro* release studies:**

The release profile of the formulations shown in Figure 1.The results indicated that the formulation F-3 showed better sustaining effect amongst all formulations. This may be due to the presence of higher concentration of cabopol 940 along with HPMC (E50LV) in the formulation F-3. The *in vitro* release profile of F-3 was then compared with marketed formulation of Lomefloxacin HCL. From the release studies, it was found that the drug release was about 24.04% and 12.49% for marketed product and F3 respectively after initial 15min. And at the end of two hours the drug release was 99.05% and 32.86% for marketed product and F-3, respectively. The comparative release was shown in fig 2. Results indicated that, the drug release was significantly prolonged by using the *in situ* gelling system due to the addition of the polymers carbopol 940 and HPMC (E50LV).

From the results it is concluded that the high viscosity plays important role in controlling the release of drug from the formulations. When the polymer concentration increases drug release decreases, and when polymer concentration decreases drug release from the formulation increases.

Antimicrobial activity:

The results of the antimicrobial efficacy tests are shown in Figure-4. The study indicated that the Lomefloxacin HCL retained its antimicrobial efficacy even after formulated as an *in situ* gelling system.

The anti-microbial activity of Lomefloxacin HCL *in situ* gel formulation may be due to a fairly rapid initial release of drug into the viscous solution formed by dissolution of gel, followed by formation of a drug reservoir that permits the drug to be released to the target site relatively slowly.

Sterility test:

The formulation CH-11, 15, 21, 25 passed the test for sterility as there was no appearance of turbidity and hence no evidence of microbial growth when incubated for not less than 14 days at 30-35 C in case of fluid thioglycolate medium and at 20 25 C in the case of soyabean casein digest medium.

Ocular irritancy studies:

The observations of ocular irritancy are shown in Table-4. The results of the ocular studies indicated that the formulation CH-11 was non-irritant with excellent ocular tolerance. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctiva were visible.

Stability studies:

From the results it has been observed that the formulations showed no change in appearance, clarity and pH. Further it was observed that the gelling capacity of the formulations was least affected.

FT-IR studies:

FT-IR spectrum of pure drug and mixture of drug and polymers are shown in Figure-5. From the spectral study it was observed that there was no significant change in the peaks of pure drug and drug polymer mixture. Hence, no specific interaction was observed between the drug and the polymers used in the formulations.

CONCLUSION

The novel ophthalmic pH-monitered *in situ* gelling drug delivery was successfully formulated by using carbopol 940 and HPMC (E4M, E50LV). The formulated *in situ* gelling systems were characterized for appearance, clarity, pH, gelling capacity, rheological character, in vitro release in simulated tear fluid. The formulation was liquid at the formulated pH (5.0) and underwent rapid gelation upon raising the pH to 7.4. The pH-triggered *in situ* gelling system showed sustained drug release over 8-h period of time. So, this formulation is an alternate to conventional eye drops to improve the bioavailability through its longer precorneal residence time and ability to sustain drug release. The patient compliance may be improved due to the decrease in frequency of drug administration.

ACKNOWLEDGEMENT

The authors are thankful to Ipca Limited, Ratlam for providing the drug samples for the study.

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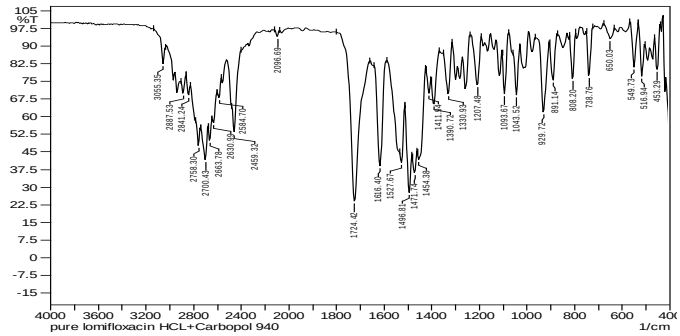
TABLES and FIGURES

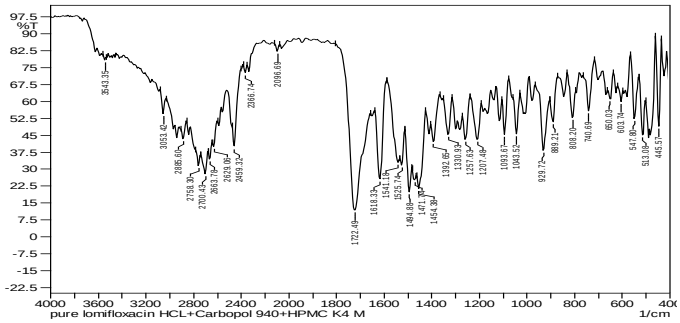
Table no 1: Formulation and Gelling Capacity Detail

Formulation Code	Concentration of Carbopol/HPMC E50LV (%w/v)	Viscosity (cps at 20 rpm)		Gelling Capacity
		pH 5	pH 7.4	
CH1	0.1/0.5	0.98	0.98	-
CH2	0.1/1.0	4.88	7.88	+
CH3	0.1/1.5	26.58	30.70	+
CH4	0.1/2.0	53.0	89.40	++
CH5	0.2/0.5	2.60	2.81	-
CH6	0.2/1.0	6.84	8.06	+
CH7	0.2/1.5	29.05	48.0	++
CH8	0.2/2.0	66.60	91.9	++
CH9	0.3/0.5	4.22	4.36	-
CH10	0.3/1.0	12.38	12.42	+
CH11	0.3/1.5	33.13	83.40	++
CH12	0.3/2.0	85.90	130.6	+++
CH13	0.4/0.5	6.42	7.69	-
CH14	0.4/1.0	17.44	21.28	+
CH15	0.4/1.5	38.68	85.50	++
CH16	0.4/2.0	98.90	147.30	+++
CH17	0.1/0.2	0.5	1.70	-
CH18	0.1/0/4	4.92	19.60	+
CH19	0.1/0.6	42.80	57.40	++
CH20	0.2/0.2	4.78	9.60	+
CH21	0.2/0.4	42.70	64.60	++
CH22	0.2/0.6	102.80	131.60	+++
CH23	0.3/0.2	22.33	76.90	+
CH24	0.3/0.4	43.50	63.20	++
CH25	0.3/0.6	111.40	145.9	+++

Table No 2: Evaluation Parameters

Evaluation steps	HL 11	CHL 15	HL21	HL 24
Visual Appearance	Transparent	Transparent	Transparent	Transparent
Clarity	Clear	Clear	Clear	Clear
pH	4.99	4.97	5.01	4.98





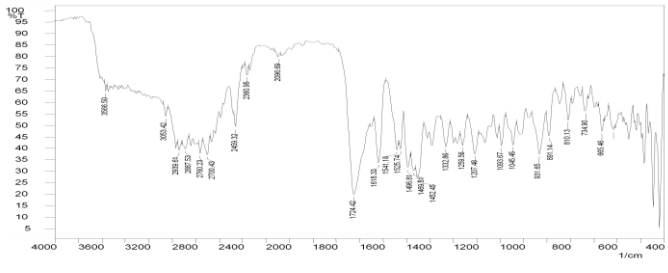


Figure 1: FTIR Data