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Sustained Ophthalmic Delivery of Levofloxacin Hemihydrate from An Ion Activated In Situ Gelling System

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Abstract: Ocular bioavailability is always poor from conventional ophthalmic drops due to spillage and nasolachrymal drainage. Ocular in situ gels can increase the drug residence time thus increasing bioavailability. Purpose of current study is to prepare sustained release Insitu ocular gels of levofloxacin hemihydrateusing gelrite as gel forming polymer, which is used in treatment of various bacterial infections. Formulations were evaluated for physical parameters like Clarity, pH, drug content, gelation, Rheological studies, sterility test, *in vitro* drug release study and ocular irritancy studies. The formulated gels were transparent, uniform in consistency and had spreadability with a pH range of 7.1 to7.4. Six different formulations with increasing polymer concentrations were prepared which was found to have drug content of 72-86%. From the preliminary studies it was observed that as the concentration of polymer was increased, the rate of drug release decreased to produce sustained drug delivery for prolonged period of more than 8 hours. A maximum of 90.2% drug release was observed in *in vitro* studies. Further *in vivo* results conclude that it is be possible to formulate in situ ocular gel containing Levofloxacin Hemihydrate.
Keywords: Gelrite, Levofloxacin hemihydrate, in situ, Gelation.

Introduction

Eye drops that are conventional ophthalmic delivery systems often result in poor bioavailability and therapeutic response, because high tear fluid turnover and dynamics cause rapid precorneal `elimination of the drug. A high frequency of eye drop instillation is associated with patient non-compliance. Inclusion of excess drug in the formulation is an attempt to overcome bioavailability problem is potentially dangerous if the drug solution drained from the eye is systemically absorbed from the nasolacrimal duct. Various ophthalmic vehicles such as inserts, ointments, Suspensions and aqueous gels have been developed in order to lengthen the residence time of instilled dose and enhance the ophthalmic bioavailability.[1] These ocular drug delivery systems however have not been used extensively because of some drawbacks such as blurred vision from ointments or low patient compliance from inserts.

Several in situ gelling system have been developed to prolong the precorneal residence time of a drug and improve ocular bioavailability. These systems consist of polymers that exhibit sol to gel phase tansititions due to change in specific physico chemical parameter (pH, temperature) in their environment, the cul-de-sac in this case. Depending on the method employed to cause sol-to-gel phase transition on the eye surface the following three types of systems are recognized, pH triggered system Eg: Carbopol, Cellulose acetate phthalate latex, temperature dependant system Eg: pluronics, tetronics, methyl cellulose, ion activated system Eg: Gelrite, Sodium alginate etc.[2]

The principal advantage of in situ gels is that they can

be easily administered with accurate and reproducible dose compared to that of preformed gels and have an advantage that they can be easily instilled in liquid form, and are capable of prolonging the residence time.[3]

Levofloxacin hemi hydrate is broad spectrum anti bacterial drug, which acts by inhibiting bacterial DNA gyrase enzyme which is required for DNA replication, gelrite is an anionic deacetylated exocellular polysaccharide secreted by Pseudomonas elodea, with a tetra saccharide repeating unit of one -L-rhamnose, one-D-glucuronic acid and two -D- glucose residues. It has the property of cation-induced and temperature dependent gelation. The aim of this study is to prepare in situ ophthalmic gel of Levofloxacin hemihydrate using gelrite to enhance ocular bioavailability and reduce dose frequency and thereby increasing patient compliance.

Materials and Methods

Levofloxacin hemihydrate was obtained as a gift sample from KAPL Bangalore, gelrite was purchased from sigma labs ltd Mumbai, and all the ingredients used were of analytical grade.

Preparation of In situ gel

Polymer solution was prepared by dispersing gelrite de ionized water by heating up to 90^0c for 20 minutes fallowed by cooling to room temperature, drug solution was prepared by dissolving levofloxacin hemihydrate in mixture of propylene glycol and water(1:0.08),drug solution was mixed with polymer solution using a magnetic stirrer , Benzalkonium chloride was added which acts as preservative. The prepared in situ gels were filled in glass vials closed with rubber closures and sealed with aluminium caps and sterilized by autoclave at 121^0c for 20 minutes.[4, 5]

Evaluation of Formulations

Appearance Formulations were examined visually for color and clarity against white background and for thepresence of particulate matter any if present.[6]

pH and Gelation studies

pH was determined by using pH meter. Gelling capacity of formulations was evaluated in order to identify the formulationssuitable for use as in situ gelling systems. Gelling was determined by mixing the formulation with simulated tear fluidin the propartion25:7 and examined visually.[7]

Composition of simulated tear fluid:

Sodium chloride	: 0.670g
Sodium bicarbonate	: 0.200g
Calcium chloride dihydrate	: 0.08g
De ionized water	: 100g

Drug Content

The vials containing formulation were properly shaken for 2-3 min. One ml of the formulation was transferred into 100 ml volumetric flask with 1 ml calibrated graduated pipette. 50 ml of simulated tear fluid with pH 7.4 was added gel was completely crushed with the help of glass road followed by vigorous shaking until the formed gel gets completely dispersed to give clear solution. Final volume was adjusted to 100 ml with STF, Aliquot of 1ml was taken and further diluted to 10 ml with STF, Obtained solution was filtered through 0.45 micron filter membrane and the drug concentration was determined by UV Visible spectrophotometer at 287.5 nm.[8]

Rheological Evaluation

Viscosity of the instilled formulation is an important factor in determining residence time of drug in the eye. The prepared solutions were allowed to gel in the simulated tear fluid and then the viscosity determination were carried out by using Brooke field viscometer RVT model with angular velocity ran from 10-100 rpm.[9] Viscosity of the formulations increased with increase in polymer concentration. The hierarchy of shear rate was reversed and average of two readings was used to calculate viscosity.

TABLE 1: Composition of Levofloxacin hemihydrate in situ gels.

INGREDIENTS	F1	F2	F3	F4	F5	F6
Levofloxacin hemihydrate	0.512	0.512	0.512	0.512	0.512	0.512
Gelrite	0.2	0.3	0.4	0.5	0.6	0.7
Propylene glycol	8	8	8	8	8	8
Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01
Water(q.s)	100	100	100	100	100	100

TABLE 2: Evaluation parameters

FORMULATION CODE	pH	Gelation	% Drug content
F1	7.12	++	78.0
F2	7.24	+++	72.9
F3	7.26	+++	77.0
F4	7.25	+++	71.0
F5	7.31	+++	84.0
F6	7.38	+++	86.0

Note: ++ gelation immediate and remains for few hours; +++ shows gelation immediate and remains for extended period.

TABLE3: In vitro release profile of In situ gels.

TIME IN HRS	% CUMILATIVE DRUG RELEASE					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
1	30.0	20.1	18.2	14.30	13.8	12.6
2	42.3	31.8	26.3	26.14	28.7	22.4
3	51.2	43.6	38.4	34.15	36.4	36.2
4	66.8	54.2	46.8	43.81	44.3	42.8
5	74.9	62.3	56.9	54.31	56.2	51.2
6	79.6	69.4	68.2	62.34	64.6	58.8
7	87.9	78.9	72.0	67.63	68.9	64.7
8	90.2	86.2	81.6	75.0	73.8	71.1

***In vitro* drug release**

In vitro release studies were carried out using bi chambered donor receiver compartment model (Franz diffusion cell). *In vitro* release of levofloxacin hemihydrate was carried out in formulations with different concentrations of gelrite using cellophane membrane. The diffusion medium 100ml of simulated tear fluid stirred at 50rpm at 37^0 C $\pm 0.5^0$ C. One end of the diffusion tube was covered by a cellophane membranae. The1 ml formulation were spread on the cellophane membrane and membrane was placed such that it just touches the diffusion medium (STF) present in receptor compartment. The drug samples were withdrawn at the interval of one hour for the period of 8 hrs from diffusion medium and analyzed by a U.V spectrophotometer at 287.5nm using simulated tear fluid as blank. [10]

Sterility Testing

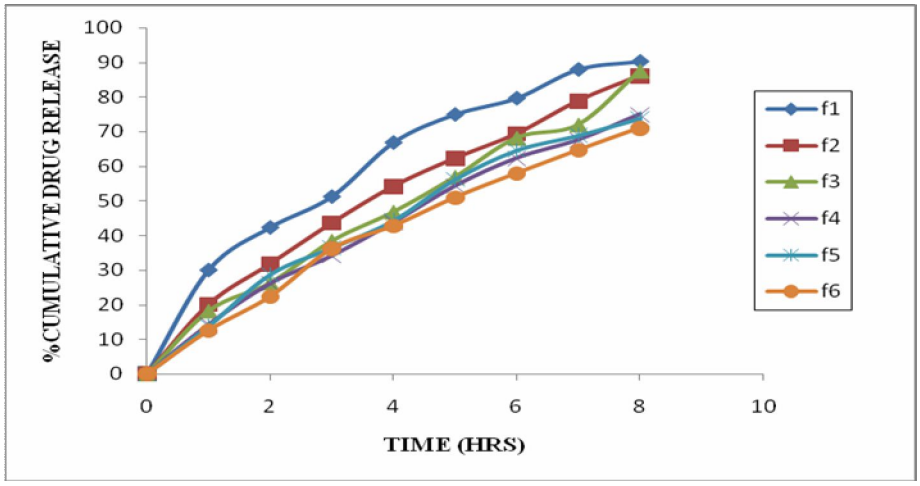
Sterility testing was performed for aerobic and anaerobic bacteria and fungi by using fluid thioglycolate and soybean caseindigest medium respectively as per the Indian Pharmacopoeia. Formulations were taken into laminar airflow and passed through a membrane filter of 0.45 µ m with the help of vacuum pump. After filtration, thefilter paper was removed from funnel and cut into two halves. One half was dropped in bacterial media (fluid thioglycolate) and other was dropped in fungal media (soybean casein digest). Both media were kept for incubation at 37^0 C for 7 days and observed for any microbial growth. The sterility test results were compared with positive and negative controls.[11]

TABLE4: Sterility test data of prepared In situ gels.

Formulation code	**Days of incubation**						
	1	2	3	4	5	6	7
F1	—	–	–	–	–	–	–
F2	–	–	–	–	–	–	–
F3	–	–	–	–	–	–	–
F4	–	–	–	–	–	–	–
F5	–	–	–	–	–	–	–
F6	–	–	–	–	–	–	–

Note: '–'sign indicates no Growth.

Figure1: % Cumulative release of Levofloxacin hemihydrate in situ gels.



FTIR Interaction studies

IR spectroscopy was performed by using Fourier transform infrared spectrophotometer (840, Shimadzu, Japan). The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi on KBr-press and the spectra was scanned in the wave number range of 4000-600 cm^{-1}. FTIR study was carried on pure drug, physical mixture of drug and polymers, formulations to confirm the compatibility of drug with other excipients used in the preparation of In situ gels.[12]

Ocular irritancy studies

The optimized formulation F6 was used for in vivo studies, the protocol was approved by college ethical committee with registration number BCP/IAEC/PCU-01.The Draize technique was designed for the ocular irritation potential of the ophthalmic product prior to marketing .[13]According to the Draize test, the amount of substance applied to the eye is normally 100µl placed into the lower cul-de-sacwith observation of the various criteria made at a designed required time interval of 1hr, 24hrs, 48hrs, 72hrs, and 1week after administration.[13, 14] Three male rabbits weighing 1.5 to 2kg were used for the present study. The sterile formulation was instilled twice a day for a period of 7 days, and a cross-over study was carried out (with a 3 day washing period with saline was carried out before the cross-over study). Rabbits were observed periodically for redness, swelling, watering of the eye.

Results and Discussions

The preparation of in situ ocular gelling system was carried out by using gelrite polymer in different Concentrations, then the formulations were subjected to general appearance, pH, gel strength, drug content, rheological studies, In vitro release studies, FTIR interaction studies, sterility test and ocular irritancy studies.

Clarity of all formulations was found to be satisfactory. The pH was within acceptable range and hence would not cause any irritation upon administration of the formulation. Table 2 also shows the result of drug content for all formulations. The drug content was found to be in acceptable Range for all formulations. Percent drug content in all six formulations were in the range 72-86 %. The two main prerequisite of gelling system are viscosity and gelling capacity. The Formulation F6 has an optimum viscosity 1985cps before gelation; which will allow its instillation into the eye as a liquid this will then undergo rapid sol-gel transition due to ion exchange and the viscosity after gelation was found to be 4520cps. Moreover, to facilitate Sustained release of drug to the ocular tissue the in situ formed gel should preserve its integrity without dissolving or eroding for a prolonged period of time. All the formulations gelled instantaneously on contact with STF. The *in vitro* release studies were carried out for all Formulations using STF as the dissolution medium. The data of these studies are present in Table 3. Results indicated that F6 showed better sustaining effect amongst all formulations. This may be due to the higher concentration of gelrite. The formulation F6 passed the sterility test as there was no appearance of turbidity and hence no evidence of microbial growth when incubated for not less than 7 days. Results of the study are indicated in Table 4. Ocular irritancy study showed there was no appearance of redness, swelling or watering when instilled in rabbit eye.

Conclusion

Levofloxacin hemihydrate which is a broad spectrum anti bacterial agent used in the treatment of ocular infections was successfully formulated as in situ gel using gelrite as polymer. The formulated systems provided sustained release of the drug for more than 8hr period. The developed formulation is a viable alternative to conventional eye drop due to its ability to enhance bioavailability through its longer precorneal residence time and ability to sustain release of the drug. Also important is its ease of administration and decreased frequency of administration resulting in better patient compliance.

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