ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 04, pp.48778-48785, April, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

FORMULATION DEVELOPMENT AND EVALUATION OF SMART POLYMER GEL FORMULATION FOR OPHTHALMIC DRUG DELIVERY

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ARTICLE INFO

Article History:
Received 23rd January, 2017
Received in revised form
08th February, 2017
Accepted 17th March, 2017
Published online 20th April, 2017

Key words:
LFX,
OFX,
NFX,
Opthalmic Drug Delivery,
and Polymer Gel.

ABSTRACT

Drug carrier can be defined as the administration of drug from natural or synthetic origin, in order to control in vivo availability of drug molecules for pharmacological effects. Rapid and efficient drainage and the relative impermeability of the cornea account for poor ocular bioavailability. To increase ocular bioavailability of drug, we need to increase ocular residence time of the drug. In situ gelling systems are viscous gelling solutions that posses phase transition on the eye due to change in certain physical and chemical properties. The purpose of the present work was to develop in situ gelling systems for fluroquinolone drugs viz. levofloxacin hemihydrate, ofloxacin (BCS Class I); and norfloxacin, equivalent to 0.5%, 0.3% and 0.3%w/v respectively by using three different mechanisms for phase transition viz. pH, ion and temperature. In situ gel forming abilities of the developed systems significantly controls precorneal drainage. Thus, increased residence time in eye would help to increase ocular bioavailability. Optimized thermosensitive in situ gelling medicated formulation containing levofloxacin hemihydrate, ofloxacin and norfloxacin was found to be well tolerated and nonirritant. The optimized systems of levofloxacin hemihydrates, ofloxacin and norfloxacin are in situ gelling and remain in the form of clear solution.

Citation: Dr. G. Pratap Kumar and Vandana Esteru Rani, 2017. "Formulation development and evaluation of smart polymer gel formulation for ophthalmic drug delivery", *International Journal of Current Research*, 9, (04), 48778-48785.

INTRODUCTION

Drug carrier can be defined as the administration of drug from natural or synthetic origin, in order to control the *in vivo* availability of drug molecules for pharmacological effects. After the application of dosage, usually a small amount remains available to the sites of interest and major fraction to the unwanted sites giving side effects. Development of drug carrier intended to reach tissues to utilize maximally giving minimum side effects. Drug carrier systems are become more difficult because of the, arrival of low-molecular-weight molecules. Also emergence of bio-macromolecules with poor aq. solubility; tissue permeation and increased use of bio-materials with less understanding of physical properties all limit research in drug carrier systems. Controlled delivery to the eye of drugs is limited by effective protective mechanisms offering by corneal tissues. Good penetration is prerequisite for absorption of drugs to the eyes and more contact time. One of the way of optimization of drug carrier system to eye is by improving precorneal drug retention. To optimize drug carrier, the various characteristics are required such as, good precorneal penetration, improved contact time, ease of instillation, non-irritative and comfortable form and optimum viscosity. An ultimate ocular carrier system would be designed as in the form of drops with no blurred vision/irritation. This would need one to three applications a day. The usefulness to patient is simplicity, a reduced frequency of instillation, minimum toxicity and untoward effects. Still existing most carrier systems are, "superficially primitive and less effective" However, one of the most facilitating and difficult target facing by the researchers is drug delivery to eye. The present investigation involves formulation development of the IS hydrogel of FQ and BCS Class I drugs, to increase residence and bioavailability. Different gelling systems viz. pH sensitive, ion sensitive and temperature sensitive are used. To develop the ISG ophthalmic solutions of FQ drugs viz. LFX, OFX and NFX, equivalent to 0.5%, 0.3% and 0.3% w/v respectively.

- **Phase transition based on temperature:** Combinations of PXM 407 and PXM 188 which undergoes transition from liquid to gel at eye temperature (33-34^{o}C) were selected to form ISG ophthalmic solutions with the aid of mucoadhesive polymer, HPMC K4M and chitosan as a penetration enhancer.
- **Phase transition based on ion:** Alginates and gellan gum are known to undergo transition from liquid to gel in presence of cations in tear fluid (Ca^{+2}, Na^{+}), hence

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selected to form ISG ophthalmic solutions with the aid of mucoadhesive polymers.

- **Phase transition based on pH:** CP are known to undergo transition from liquid to gel in the presence of higher pH of tear fluid, hence were selected to form ISG ophthalmic solutions with the aid of mucoadhesive polymer, HPMC K4M. CP 974P was used as it is a benzene free grade of CP.
- Evaluation of developed ISG formulations for appearance, clarity, gelation temperature, gelling capacity, pH, drug content, *in vitro* drug release, transcorneal permeation study, mucoadhesion, antimicrobial efficacy and isotonicity.
- Further, evaluation of optimized formulations for ocular irritation, ocular pharmacokinetic study, precorneal clearance study using gamma

Plan of work

- **Literature survey**
- **Collection of drug, polymers and other excipients**

1. Preformulation studies
2. Characterization of drugs and polymers
3. Selection of vehicle
4. Formulation developments

IV) Evaluation

- Appearance and clarity
- pH
- Gelation temperature
- Gelling capacity
- Drug content
- Mucoadhesion test
- *In vitro* drug release studies
- Rheological behavior
- Transcorneal penetration studies
- Drug polymer interaction studies
- Antimicrobial efficacy studies
- Isotonicity
- Precorneal clearance study using gamma scintigraphy
- Ocular irritation studies
- Ocular pharmacokinetic study by HPLC
- Stability studies

EXPERIMENTAL

Preformulation studies

Preformulation studies were performed for the obtained sample of LFX, OFX and NFX for characterization and compatibility studies.

Characterization of drugs

A) Appearance

The samples of individual drug viz. LFX, OFX and NFX were observed visually using black and white background.

B) Solubility

The solubility of individual drug was tested in various solvents, like distilled water, alcohol and buffers.

C) Thermal analysis

1. Melting point

Melting point of individual drugs viz. LFX, OFX and NFX was determined by capillary method. Observed value was compared with reported value.

1. Differential scanning calorimetry (DSC)

DSC curves of individual drug were obtained in a Mettler Toledo DSC 822 cell using aluminum crucibles. About 2mg of samples, under dynamic N_2 atmosphere (flow rate: 50 mL/min) and at a heating rate of 10°C/min in the temperature range 25-400°C was analyzed.

D) Spectral analysis

i) Infrared spectroscopy

The infrared spectrum of individual drug was recorded by potassium bromide dispersion technique using FTIR with diffuse reflectance attachment (FTIR-8400s). The spectrum was obtained in the range of 400-4000cm^{-1}.

UV Spectroscopy

The stock solution of concentration 100µg/ml was prepared by dissolving 10mg of individual drug in 100 ml simulated tear fluid. After giving sufficient dilution with simulated tear fluid, each solution was kept in cuvette of path length 10mm and UV spectrum was recorded using double beam UV-VIS spectrophotometer in the wavelength range 200-400nm keeping simulated tear fluid as a blank.

Compatibility studies

Differential scanning calorimetry study and FTIR spectroscopy was carried out to check the suitability between drugs and polymer. IR spectrum and DSC thermogram of physical mixtures were compared with that of the pure drug.

Selection of vehicle

Solubility of individual drug viz. LFX, OFX and NFX was tested in various buffers such as acetate buffer I.P. pH 4.0, 4.4, 5, 5.5 and 6; citrophosphate buffer B.P. pH 6.0; and phosphate buffer USP pH 5.5, 6, 7.2 and 7.4. Solutions of LFX, OFX and NFX in the above buffers were prepared as 0.5, 0.3 and 0.3% w/v respectively.

Standard calibration curve

Stock solution of LFX, OFX and NFX was prepared by dissolving 10 mg of individual drug in 100ml of STF to get the stock solution of 100µg/ml respectively. From this stock solution, aliquots of 1, 2, 3, 4, 5, 6 and 7ml were withdrawn and further diluted to 50 ml with STF to obtain a concentrations range of 2 to 14µg/ml respectively. A graph of concentration vs. absorbance was plotted to get a standard calibration curve of individual drug respectively**.**

Formulation development

The ISG ophthalmic solutions of FQ drugs viz. LFX, OFX and NFX, equivalent to 0.5%, 0.3% and 0.3% w/v were prepared respectively. Suitable amount of different gelling agents, tonicity adjusting agent and antimicrobial preservative were added. The formulations were based on the use of three different mechanisms for phase transition from liquid to gel.

Phase transition based on temperature

Combinations of PXM 407 and PXM 188 which undergoes transition from liquid to gel at eye temperature (33-34^{o}C) were selected to form ISG ophthalmic solutions with the aid of mucoadhesive polymer, HPMC K4M and chitosan as a penetration enhancer.

PXM 407 and PXM 188 and HPMC K4M
PXM 407, PXM 188, HPMC K4M and chitosan

Phase transition based on ion

Alginates and gellan gum are known to undergo transition from liquid to gel in the presence of cations in tear fluid (Ca^{+2}, Na^{+}), hence selected to form ISG ophthalmic solutions with the aid of mucoadhesive polymers.

Sodium alginate and HEC/ HPMC K4M
Gellan gum

Phase transition based on pH

CP are known to undergo transition from liquid to gel in the presence of higher pH of tear fluid, hence was selected to form ISG ophthalmic solutions with the aid of mucoadhesive polymer, HPMC K4M. CP 974P was selected as it is a benzene free grade of CP.

CP 974P and HPMC K4M

ISG ophthalmic placebo formulations

Phase transition based on temperature

Different combinations of placebo formulations for different ISGS were developed, to determine the contents precise for application as medicated ISGS. Chitosan was dissolved in a mixture of glacial acetic acid and sterile water. Mucoadhesive polymer, namely HPMC K4M was dispersed in chitosan solution. PXM 407 and PXM 188 were slowly added to above cold mixture with continuous mixing. The partially dissolved PXM solutions were stored in a cold place for one day and stirred. Placebo formulations were evaluated for appearance, clarity, gelation temperature and gelling capacity

Formulations of PXM 407 and PXM 188 combinations

Sr. No.	PXM 407	PXM 188	Chitosan	HPMC K4M	Sterile water
		(%w/v)			
1	15	4	-	-	q.s.
2	16	4	-	-	
3	17	4	-	-	
4	18	4	-	-	
5	18	4	-	0.2	
6	18	4	-	0.4	
7	18	4	0.25	0.2	
8	18	4	0.5	0.2	

Phase transition based on ion

Sodium alginate and HPMC K4M or HEC

The gels were obtained by dispersing different concentrations of sodium alginate individually and in combination with mucoadhesive polymers viz. HPMC K4M or HEC in sterile water. Different placebo formulations were evaluated for appearance, clarity and gelling capacity

Formulations of sodium alginate and mucoadhesives

Sr. No.	Sodium alginate	HPMC K4M	HEC	Sterile water
		(%w/v)		
1	1	-	-	q.s.
2	1.5	-	-	
3	2	-	-	
4	1.5	0.2	-	
5	1.5	0.4	-	
6	1.5	-	1	
7	1.5	-	1.5	
8	1.5	-	2	

Gellan gum

Appropriate quantities of gellan were dispersed in sterile water. The dispersions were heated to 90^{o}C for 20 min while stirring and allowed to cool at room temperature. Different placebo formulations were evaluated for appearance, clarity and gelling capacity

Gellan gum ISGS

Sr. No.	Gellan gum (%w/v)	Sterile water
1	0.1	q.s.
2	0.2	
3	0.3	
4	0.4	
5	0.5	

Phase transition based on pH

Appropriate quantities of CP 974P and mucoadhesive polymer, HPMC K4M were dispersed in citrophosphate buffer pH 6.0; the gels were allowed to swell overnight. Different placebo formulations were evaluated for appearance, clarity, pH and gelling capacity

Formulations of CP 974P and HPMC K4M

Sr. No.	CP 974P (%w/w)	HPMC K4M	Citrophosphate buffer pH 6
1	0.1	1	q.s.
2	0.2	1	
3	0.3	1	
4	0.4	1	
5	0.5	1	

ISG medicated formulations

For preparing ISG medicated formulations, FQ drugs viz. LFX, OFX and NFX, equivalent to 0.5%, 0.3% and 0.3% w/v were dissolved in sterile water with the aid of 0.1N HCl or 0.1N NaOH to get clear drug solution. Further these drug

solutions were mixed with previously optimized ISGS. Mannitol (5%w/v) as Isotonicity agent and BAC (0.01%w/v) or methyl paraben (0.1%w/v) as preservative, were added to all formulations. Finally pH of formulations was adjusted. Formulations were autoclaved at 15 psi pressure for 20 min. Apparatus used during the formulation development of the ISGS were autoclaved and the complete work was carried out in aseptic area.

Phase transition based on temperature

Formulations of 18% w/v PXM 407 and 4% w/v PXM 188

Formulation code	LFX	OFX	NFX	Chitosan	HPMC	Sterile
					K4M	water
			(%w/v)			
P1	0.5	-	-	-	0.2	q.s.
P2	0.5	-	-	-	0.4	
P3	-	0.3	-	-	0.2	
P4	-	0.3	-	0.25	0.2	
P5	-	0.3	-	0.5	0.2	
P6	-	-	0.3	-	0.2	
P7	-	-	0.3	0.25	0.2	
P8	-	-	0.3	0.5	0.2	

Evaluation

Appearance and clarity

All the developed formulations were observed carefully for colour and presence of suspended particulate matter if any. The clarity of solutions was further assessed by observing them against a dark and white background. Formulations were graded as follows: (-) turbid, (+) slightly turbid, (++) clear solution, (+++) clear and transparent.

pH

The pH of ophthalmic formulation should be such that the formulation will remain stable at that pH and at the same time there would be no irritation to the patient upon instillation. Ophthalmic solutions should be formulated in a pH range of 5 to 7.4.

Gelation temperature

This study was done by increasing the temperature of the formulation content in a glass test tube slowly. Two ml of formulation was placed and temperature is raised with stirring until became gelled. Gelling was optimized when there was no movement inside, when the test tubes were rotated at right angle

Gelling capacity

This was evaluated by taking one/two drop volume of the solution in a tube containing artificial tear fluid [94] and just observing the gelling and the required time for gelation. The time required for the gel formation to break was also noted.

Drug content

The content of all the ISG medicated formulations containing LFX, OFX and NFX was determined by diluting 1 ml of formulation to 50 ml freshly prepared STF, pH 7.4. The volume was made up to the mark ie.(100 ml) with freshly prepared STF. The solutions were filtered through filter membrane (0.45-mm) and concentrations were determined at λ_{max} by using UV-Vis spectrophotometer.

Mucoadhesion test

All the ISG medicated formulations containing LFX, OFX and NFX were evaluated by the process described [144]. The mucoadhesive strength of ocular gels was determined by means of the mucoadhesive force measuring assembly, using goat corneal membrane. The diameter of corneal membrane was 1.1cm. The vials with the corneal membrane were stored at 37^{o}C for 10 min. One vial with a corneal membrane was attached to the balance and the second vial was fixed on a length adjustable pan. The gel was added on the first vial membrane surface. Then, the height of the vial was adjusted. Membrane surfaces of both vials should come close. A contact time of 8-10 minutes was allotted. Then, the load was allowed to increase in the pan until vials seperation. The bioadhesive force was the least load required to separate two vials. It expressed as the detachment force (dyne/cm^2).

Detachment stress (dyne/cm^2) = m×g/A

Where,
m = the weight added to the balance in grams.
g = acceleration due to gravity taken as 980 cm/sec^2.
A = area of corneal membrane exposed and is equal to r^2.
For measurement of mucoadhesive strength of gel formulation a modified balance assembly was used.

In vitro drug release studies

In vitro drug release of all ISG medicated formulations containing LFX, OFX and NFX was performed. The medium used was freshly prepared simulated tear fluid (pH 7.4). Previously soaked cellulose membrane was tied to one end of glass cylinder. Specific amount of the formulation was introduced into the glass tubes. Then the glass cylinders were attached to the metalic rotating shaft and dipped in 100 ml of medium maintained at 34±1^{o}C. The shafts were rotated at 50 rpm. At time intervals of 1hr, samples were withdrawn and replaced to maintain sink conditions. The drug content was determined at the λ_{max} by using UV-Vis spectrophotometer. The results were the averages of three runs.

Rheological behavior

Rheological behavior of all the selected ISG medicated formulations (solution) containing LFX, OFX and NFX was performed. The specific quantity was added into the small sample holder (Brookfield viscometer LVDV-II + Pro model) with T- bar spindle code S 93. Run involved changing the velocity from 1 to 100 rpm at a controlled speed which was changed after every 10 sec. (1, 5, 10, 20, 50 and 100) rpm.

Transcorneal permeation studies

A device designed by Gonjari *et al.* was used to evaluate drug permeation through a goat corneal membrane of all the selected ISG medicated formulations containing LFX, OFX and NFX. Eyeballs of sheep were obtained and maintained in routein saline at 4^{o}C. The washed corneas were kept in cold and fresh prepared simulated tear fluid (pH 7.4) and placed on by arranging in between the clamped donor and receptor

compartment. Simulated tear fluid was used as a diffusion medium. The ISG formulations and marketed eye drop solution of LFX, OFX and NFX each was added to the donor compartment. The membrane surface was in contact with simulated tear fluid. A temperature of 34 ± 0.5°C was maintained. A magnetic stirrer in the cell provided continuous agitation. At appropriate time intervals of entire process, one ml of sample was collected and replaced with medium to maintain sink conditions. The absorbance was measured at λ_{max} by using UV-Vis spectrophotometer. The results were the means of three runs. Drug release data was fitted to zero-order, first-order, Higuchi and Korsmeyer-Peppas

Drug polymer interaction studies

Interaction studies investigated effect of autoclaving on the properties of the selected ISG medicated formulations. As ophthalmic formulations must be sterile, all ISG medicated formulations were sterilized by the moist heat sterilization using autoclave at pressure 15 psi for 20 min. Parameters like appearance, clarity, pH, gelling ability, drug content and gelation temperature were studied after autoclaving. Also thin layer chromatogram of pure drug solution and optimized formulation was taken after autoclaving. Thin layer chromatogram of pure drug and optimized formulation was obtained using a solvent system consists of n-butanol, methanol and ammonia in the ratio of 5:1:1.5

Antimicrobial efficacy studies

Antimicrobial efficacy was determined by the agar diffusion test employing Boar well method. Marketed eye drop solution as standard and ISG medicated formulations containing LFX, OFX and NFX were inserted into wells bored into sterile nutrient agar seeded with test organisms (*Staphylococcus Aureus and Pseudomonas Aeruginosa*). Passage of the solutions for 120 minutes was permitted. Agar plates were incubated at 37°C for 24 hr. The zone of inhibition (ZOI) was measured of each well and compared with standard. The entire operation excluding incubation was carried out in laminar air flow

Isotonicity

Isotonicity is prerequisite property of the various eye preparations. Isotonicity should be checked for any tissue damage or irritation of eye. The ISG medicated formulations containing LFX, OFX and NFX were subjected to Isotonicity testing. Blood was collected with the help of haemocytometer and formulations were mixed with drops of blood and examined under microscope at 45X magnification. Later comparison with standard marketed LFX ophthalmic formulation was done.

Precorneal clearance study using gamma scintigraphy

In vivo precorneal clearance of radionuclide was studied using single photon emission computing tomography (SPECT LAB). Six IS gel formulations as well as marketed eye drops were assessed in terms of their ocular retention time. Four New Zealand Albino rabbits weighing 2-2.5 ± 0.5 kg were used for the study. Both, thermosensitive and gellan based ion sensitive gelling formulations containing LFX, OFX and NFX were assessed on a group of four rabbits with a washout period of 3 days. Each drug was radiolabeled with a reducing agent (Tc-99m), by direct labeling method. Rabbits were anaesthetized using ketamine HCl injection given intramuscularly in a dose of 15 mg/kg body weight. The rabbits were positioned 5 cm in front of the probe and 25µL of the radio labeled formulation (equivalent to ~100 µ ci) was instilled onto the left corneal surface of the rabbits. Recording was started immediately after instillation at a rate of 15 seconds per image for 10 min. and more using SIEMENS ECAM gamma camera (SPECT Lab Pune, India). Region of interest (ROI) was selected. Time activity curve was plotted to calculate the rate of drainage from eye upto 10 min. A single whole body static image also was taken after 2 hr of instillation of formulation.

Evaluation of ocular irritation

The procedure was performed on three New Zealand white albino rabbits, each weighing 2–3 kg. 100ul of optimized thermosensitive ISG medicated formulations containing LFX, OFX and NFX was instilled into the lower *cul-de-sac* of the left eye of the rabbit. The right eye, kept untreated to serve as a control. To prevent loss of instilled volume, eye lids were held together for app. 5 sec. The sterile formulations were instilled three times a day and the rabbits were observed after 1 hr, 24 hr, 48 hr, and 72 hr for redness, excessive tearing, and inflammation of the eye.

Animal house keeping

Four healthy New Zealand white rabbits (2-2.5 kg) were purchased from National Toxicology Centre (NTC), Pune. The animals were housed under standard laboratory conditions of temperature (22±1°C) and 12/12 hr light / dark cycle. They were fed with fresh leaves of cabbage and cauliflower and water ad libitum. All the experiments were approved and conducted as per guidelines of Institutional Animal Committee.

Quarantine

The experimental animals were quarantined in a well ventilated room with controlled humidity and lighting.

Ocular pharmacokinetic study

New Zealand white rabbits of about 5-6 months age and weighing about 2-2.5 kg were selected. Eyes of the rabbits were examined before start of procedure for presence of any abnormality. All the procedures conducted as per guidelines of the OECD principles of good laboratory practice strictly. Each optimized thermosensitive IS gelling medicated formulation containing LFX, OFX and NFX and marketed eye drop formulations of each drug were assessed on a group of four rabbits. Four rabbits were divided into four groups. Each group received a topical administration of P1, P4, P7 and marketed eye drops respectively with a minimum washout period

Procedure

50 µl of medicated formulation was instilled into the lower *cul-de-sac* of left eye of the rabbit. The right eye, which remained untreated, served as a control. The conjuctival sac was held for 30 sec. with the help of fingers. Rabbits were anaesthetized. Ketamine HCl injection IM, in a dose of 15 mg/kg body weight was used and aq. humor was sampled with the help of 28 G needle after 30 min, 1hr, 2hr, 3hr, 4 hr, 5 hr

and 6 hr from left eye. 100 µl of Aq. humor was mixed with methanol (1:1) and kept in a refrigerator for 60 min. The mixture was then centrifuged at 3000 rpm for 15 minutes. 20 µl of the supernatant was quantified for drug content by HPLC

Calibration curve

A stock solution of each drug (1.0 µg/ml) was prepared by dissolving an appropriate amount of drug in methanol. Working standard solutions of each drug were prepared daily by dilution of stock solution with methanol. To prepare the aq. humor calibration standards, aliquots of 50 µl of aq. humor were placed in each Eppendorf tube and spiked with increasing concentrations of working standard solutions of each drug to give concentrations of 100, 200, 300, 400 and 500ng/ml. Calibration standards were processed according to sample preparation procedure and were analyzed by HPLC method.

The chromatographic conditions followed were as follows:

HPLC Unit : Cyberlab MAO1527, USA
Pump : Cyberlab high pressure gradient mixer
Detector : Cyberlab UV/VIS detector
Column :C-18 (250 mm length x 4.6 mm internal diameter and 5 µm particle size, Make-SMT SAM)
Mobile Phase : Methanol: Water (50:50) pH adjustment with glacial acetic acid.
Flow rate : 1 ml/min
Loop size : 25 µl
Detection : 295 nm for LFX; 295 nm for OFX and 278 nm for NFX.

Data Analysis

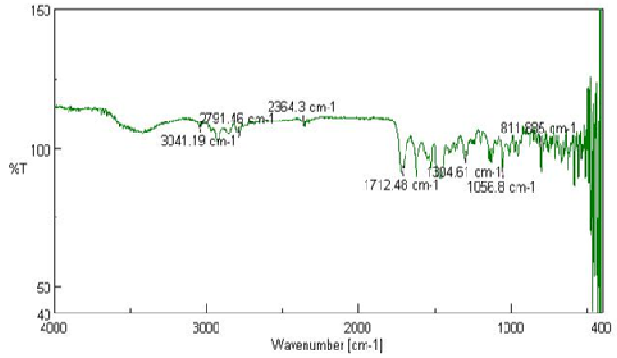
Pharmacokinetic parameters were determined by non-compartmental analysis. The maximum concentration of drug in the aq. humor (C_{max}) and the time required to reach the maximum concentration (T_{max}) were obtained from the aq. humor drug concentration versus time curves. The area under the aq. humor concentration versus time curve (AUC) was calculated by trapezoidal rule. The rate constant (k) was calculated by log linear regression of the last data points (terminal portion) of the aq. humor concentration versus time curve. The half-life was calculated from the equation $t_{1/2} = 0.693/K_{el}$.

Stability studies

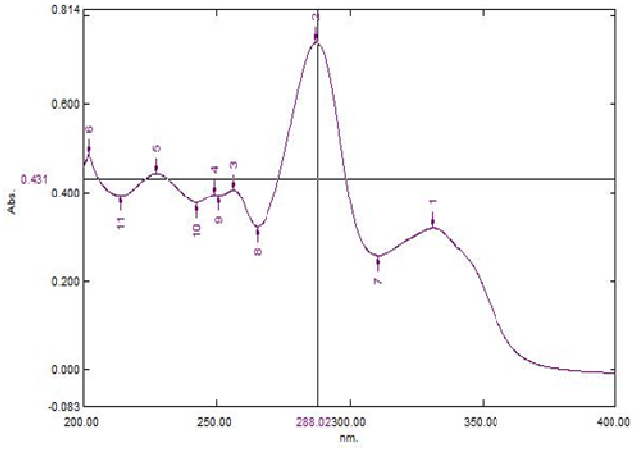
The stability testing requirements for various types of ophthalmic products (eye drops, eye ointments, ophthalmic inserts, injections, irrigating solutions, lens care products are not always that simple. The International Conference on Harmonization (ICH) guidelines does not address all of the stability requirements for the vast variety of products. For the large number of ophthalmic formulations that are packaged in semi-permeable containers, “stress conditions” are present at high temperatures and low humidity. Thus, accelerated testing of these products is carried out under these conditions as per ICH guidelines. The specific conditions include long-term stability testing at 25°C/40%RH; intermediate accelerated (if 40°C fails) testing at 30°C/40% RH (FDA guidelines) or 30°C/60%RH (ICH guidelines) and accelerated testing at 40°C/15%RH.

Stability Protocol

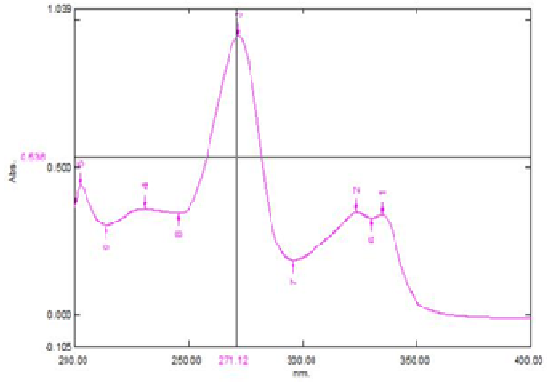
Selected sterilized formulations were stored at room temperature 5±3°C and 30±2°C/65% RH ±5% RH for a period of 3 months. The formulations were evaluated at predetermined intervals for drug assay, clarity, pH, liquid–gel conversion and transcorneal permeation profile. The rate constant of decay was determined by plotting the log of % drug remaining vs time for P1, P4 and P7 respectively using Arrhenius plot according to first order kinetics. The degradation rate constants were calculated from slopes of the straight lin



IR spectra of OFX sample



Spectra of LFX in simulated tear fluid



Spectra of NFX in simulated tear fluid

Standard calibration curve

The absorbance of LFX, OFX and NFX standard solutions in simulated tear fluid. The curve was found to be linear at λ_{max} of

288 nm, 293nm and 271nm resp. in simulated tear fluid. The calculation of the drug content, *in vitro* drug release and stability studies are based on this calibration curve.

Preparation of calibration curve data of in STF

LFX		OFX		NFX	
Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
0	0	0	0	0	0
2	0.150	2	0.222	2	0.232
4	0.299	4	0.362	4	0.425
6	0.419	6	0.531	6	0.641
8	0.544	8	0.694	8	0.867
10	0.709	10	0.857	10	1.027
12	0.828	12	1.013		
14	1.028				

Summary

Developed ISGS for FQ drugs viz. LFX, OFX (BCS Class I); and NFX, equivalent to 0.5%, 0.3% and 0.3% w/v were found to be promising. Suitable amount of different gelling agents, tonicity adjusting agent and antimicrobial preservative were added. The formulations were based on the use of three different mechanisms for phase transition from sol to gel viz. pH sensitive, ion sensitive and temperature sensitive are used. The results of compatibility study indicate no interaction between drug and polymers. All the developed IS gel formulations were evaluated for appearance, clarity, gelation temperature, gelling efficiency, pH and mucoadhesive force. The contents, clarity, and pH of the formulations were found to be in acceptable range and the formulations were liquid at room temperature and refrigeration too. On the basis of clarity, gelling capacity, bioadhesion force and *in vitro* drug release study of all the formulations, selected formulations viz.P1, S2, G3, C3, P4, S4, G5, C4, P7, S7, G7 and C7 were further subjected to rheological behavior and transcorneal permeation study. Thin layer chromatography was carried out to check the drug polymer interaction after autoclaving for selected formulations. Rf values were found to be nearly same for standard drug and its formulation after autoclaving. Formulations shows shear thinning nature with increase in shear stress with increase in angular velocity. The results obtained from the rheological study of prepared ISGS suggested that the viscosity decreases in ascending order of the angular velocity. The viscosity was directly proportional to concentrations of polymers present in the formulations. Higuchi matrix diffusion mechanism was observed from all ISG formulation. The release of drug from this matrix is regulated by diffusion/erosion. The overall release was diffusion-controlled. The best fit kinetic model was Higuchi matrix model. When compared using student t test ANOVA followed by Dunnet's test was done to study transcorneal permeation after 2 hr. LFX and NFX IS gel formulations showed sustained release as compared to marketed eye drop. For OFX containing IS gel formulation, only P4 showed sustained release.

Amongst the four formulations developed based on three systems, for FQ drugs viz. LFX, OFX and NFX for further study two systems were considered viz. gellan based ion sensitive ISG system and PXM based thermosensitive ISGS. The formulations were found to be isotonic when compared with marketed LFX eye drop. The result of antimicrobial efficacy study shown that there were no changes in the antimicrobial activity of FQ drugs viz. LFX, OFX and NFX due to formulation ingredients and working conditions as compared to reference formulation (marketed eye drop formulation). For scintigraphic studies, the observation of the gamma camera images showed that both, developed thermosensitive and gellan based ion sensitive ISGS form good clear gel over the corneal surface immediately after administration. Marketed eye drop solutions were cleared very rapidly from the corneal region whereas; all ISGS were cleared at slow rate and showed good retention for longer duration. IS gel forming abilities of the developed systems significantly controls precorneal drainage. Thus, increased residence time in eye would help to increase ocular bioavailability. Superficial cornea1 opacity has been observed with gellan based systems on the rabbit eye after gamma scintigraphy study. Thermosensitive ISG system does not show any opacity on the rabbit eye.

Further optimized thermosensitive ISG medicated formulation containing LFX, OFX and NFX was found to be well tolerated and nonirritant. Combination of 18%w/v PXM 407 and 4%w/v PXM 188 with 0.2%w/v HPMC K4M and 0.25%w/v chitosan, showing mucomimetic properties as well as optical clarity. The optimized IS gel formulation and commercial eye drops were subjected to *in vivo* studies to determine drug concentration in aq. humor in the eyes of rabbits. The MIC_{90} of drug in aq. humor was achieved by ISGS and remained upto study duration of 6 hr. In the marketed eye drops solution, initial increase in drug concentration was drop down after some time. C_{max} of ISG formulation was found to be higher than marketed eye drops solution at the similar T_{max} of 1 hr. C_{max} of ISG formulations i.e. P1, P4 and P7 was found to be 1.6, 1.5 and 1.3 times higher than marketed eye drops solution respectively at the similar T_{max} of 1 hr. The $AUC_{0\text{-}360min}$ of LFX is more than OFX and NFX. The results indicate the significant permeation of LFX than OFX and NFX. Also the IS gel formulation showed more AUCthan their respected marketed eye drop formulations. The more $AUC_{0\text{-}360min}$ of IS gel formulations is because of increased contact time in the eye. The developed IS gel formulations improved contact time, there by improved bioavailability of drug as proved from high drug aq. humor concentrations. Selected sterilized formulations viz. P1, P4 and P7 were stored at 5±3^{o}C and 30±2^{o}C/65% RH ±5% RH for duration of 90 days. The formulations were evaluated at predetermined intervals for assay, clarity, pH, liquid–gel conversion and transcorneal permeation, no significant change was observed. The optimized systems of LFX, OFX and NFX are ISG based on thermogelation, gels at 33-34^{o}C. The formulation should be stored at cool conditions or below 25^{o}C. At these storage conditions (cool place) the developed systems remains in the form of clear solution. As degradation was found to be less than 5 percent, approximate shelf life of 24 months can be alloted to the optimized formulations.

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