Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 4, 2011

Research Article

A NOVAL THERMOREVERSIBLE PHASE TRANSITION SYSTEM WITH FLUX ENHANCERS FOR OPTHALMIC APPLICATION

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Received: 7 Aug 2011, Revised and Accepted: 17 Sep 2011

ABSTRACT

The objective of this study is to investigate effect of flux enhancing polymers on invitro release through thermo sensitive gelling solution. Poloxamer 407 a triblock copolymer exhibiting the phenomenon of reverse thermal gelation used with different polymers including HPMC and chitosan resulting in sustained delivery of drug. Addition of PVA (0.2%w/v – 0.3% w/v) to the gelling systems shows significant increase in the percentage drug release and prolong the residence time. Phase transition temperature of these systems ranged from 28.0–39.80°C depending on the ratio of Poloxamer 407 and chitosan. The formulation consisted of 20% Pluronic and 0.2% chitosan and PVA 0.3%w/w, with the highest release efficiency (49.61 ± 0.14%) may be suggested as a suitable ophthalmic preparation for sustained release of ciprofloxacin. Release rate of the system composed of P407/chitosan/ PVA (20/1/0.3%, w/v respectively) was measurably larger than that without PVA. Viscosity of the formulation was in a suitable range at 25°C and pseudo plastic behavior was found to be at 35°C. Antimicrobial effect of the solutions was studied in nutrient agar in comparison to marketed solutions of ciprofloxacin using *Staphylococcus aureus* by the agar diffusion test using the cup-plate technique. The zone of inhibition for both studied bacteria was significantly greater for tested formulation than the marketed eye drop of ciprofloxacin. The formulation exhibited an 8-hour sustained release of CFX (ciprofloxacin).

Keywords: Poloxamer, Ciprofloxacin, Gelling solution,PVA

INTRODUCTION

Various approaches have been tried towards the development of stable sustained release in-situ ophthalmic gel so as to overcome problems associated with conventional ophthalmic dosage form. Conventional liquid ophthalmic drops exhibit a short pre-corneal residence time and poor bioavailability due to rapid and extensive elimination of drugs from pre-corneal lachrymal fluid by solution drainage, lachrymation, and non-productive absorption by conjunctiva[1, 4]. In situ gel system is formulated as liquid preparation suitable to be instilled into eyes which upon exposure to the physiologic environment changes to gel results in in-situ gel, thus increasing the precorneal residence time of the delivery system, and enhances the ocular bioavailability of the drug. But Cumulative percentage drug release from such in situ gel was found to be unsatisfactory. There is a need to study effect of various polymers on drug release rate, to develop a new ophthalmic gelling system that undergo thermoreversible phase transition and delivers the drug with highest release efficiency once administered in the cul-de-sac[5]. Ciprofloxacin HCl was used as a model drug to study the effect of various polymers on *invitro* drug release. Ciprofloxacin is a synthetic fluoroquinolone antibiotic with a broad spectrum antimicrobial activity.

The topical dosage of ciprofloxacin HCl eye drops is one to two drops of 0.3% solution in the affected eye every 4 h or hourly once in the case of severe infection . One of the major drawbacks of an antibiotic eye drops is the pulsatile drug level, with a transient period of overdose followed by an extended period of subtherapeutic levels before the next dose is administered[5]. In development of thermoreversible phase transition system, Poloxamer 407 or Pluronic F 127 was used in different concentration so that it should always show phase transition at physiological temperature when use alone and in combination with different polymers. Poloxamer 407 was unable to show any phase transition when used at or below 18% w/w[2].

The different gelling solutions were made with poloxamer in combination with different polymers such as HPMC, Chitosan and were optimized considering phase transition temperature, physical appearance, gelling capacity. On the basis of parameters as mentioned above the formulation were optimized and effect of flux enhancers on in vitro drug release for each optimized formulation was studied.

MATERIALS AND METHODS

Materials

Poloxamers P407 was obtained from BASF Corp. (Ludwigshafen, Germany), Ciprofloxacin was kindly gifted from Inventia healthcare Pvt. Ltd. (INDIA). Chitosan and Polyvinyl alcohol (PVA) were purchased from MERK. Triethanolamine, Benzalkonium chlorides were obtained from Research lab fine chem industries (INDIA), all other chemicals used were commercially available products of analytical grade.

Preparation of Formulations

Selection of vehicle

Poloxamers (trade name Pluronic®), ABA triblock copolymers consisting of hydrophilic polyoxyethylene (PEO) and hydrophobic polyoxypropylene (PPO) units, are known for exhibiting the phenomenon of reverse thermal gelation under certain concentrations (critical micellization concentration) and temperatures (critical micellization temperature) [1, 6,7]. At a concentration of 18% (w/w) or higher in an aqueous solution, poloxamer 407 (P407) is transformed from a low-viscosity solution to noncross-linked hydrogel upon being exposed to ambient temperature. Considering the lachrymal fluid dilution, a relative higher polymer concentration is essential for the P407 solution to form gel under physiological conditions[7].

Sample preparation

Table 1 shows the different composition of gelling solution of ciprofloxacin HCl. The formulations were prepared on a weight/weight basis using the cold method. In case of poloxamer certain volume of distilled water was cooled down to 4°C. An appropriate amount of P407 was then slowly added to the cold water with continuous stirring. The dispersions were stored in a refrigerator at 4°C over night resulted in clear solution [7]. Gelling solution based on poloxamer and Chitosan were prepared as follows, chitosan was initially dissolved in a solution of acetic acid (0.5% v/v) and used as a solvent for the Poloxamer dispersion [8]. In case of poloxamer and HPMC, appropriate amount of HPMC was dissolved in warm water and mixed with poloxamer solution previously refrigerated overnight. For preparation of drug-containing polymer solutions, Ciprofloxacin was dissolved in 0.5 N NaOH solution and incorporated manually

into the poloxamer mixture until homogeneity was attained. Required amount of NaCl was added to all the formulation to make them isotonic. 0.01% (w/v) benzalkonium chloride added as preservative. The samples were then transferred into amber ointment jars after adjusting the pH to 6 and stored at 4°C prior to further analysis[9]. The conventional commercial eye drop bottle delivers an average drop volume of about 40 µL, whereas the available tear fluid is 7 µL. To understand the in vivo phase transition process of thermosensitive gels, the rheological parameters were measured as a function of temperature before and after the poloxamer formulations diluted by simulated tear fluid (STF) at a ratio of 40:7. The composition of STF was sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride·$2H_2O$ 0.008 g, and bidistilled water q.s. 100 g[7].

Table 1: Composition of the Ophthalmic Gels of Ciprofloxacin HCl

Formula Variation(%w/w)	CFXF1	CFXF2	CFXF3	CFXF4	CFXF5	CFXF6
Ciprofloxacin HCl	0.30%	0.30%	0.30%	0.30%	0.30%	0.30%
Poloxamer	20%	20%	20%	20%	20%	20%
HPMC	---	0.25%	---	---	---	---
Chitosan	---	---	0.25%	0.25%	0.25%	0.25%
PVP	---	---	---	0.20%	---	---
PVA	---	---	---	---	0.20%	0.30%
NaCl	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%
Benzalkonium Chloride	0.001%	0.001%	0.001%	0.001%	0.001%	0.001%
Purified water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Determination of visual appearance, clarity, and pH

The appearance and clarity were determined visually under fluorescent light against black and white background in well lit cabinet. The pH was measured for each formulation by dispersing 2.5 gm in 25ml of purified water using a pH meter (Rolex) which was calibrated before use with buffered solution at pH 4 and 7.

Uniformity of drug content

Drug content of ciprofloxacin formulations was determined by dissolving an accurately weighed quantity of formulation (1 g) in 50 ml of pH 6 phosphate buffer. The solutions were then filtered through 0.45 m membrane filter and analyzed for ciprofloxacin content by UV spectrophotometer at 272.4 nm.

Measurement of phase transition temperature

A transparent vial containing 10 mL of ciprofloxacin gelling solution and a magnetic bar was placed in a low-temperature water bath. A thermometer with an accuracy rating of 0.1°C was immersed in the ciprofloxacin gelling solution. The solution was heated at the rate of 1°C/1–2 min with constant stirring of 5 rpm. The temperature at which the magnetic bar completely stopped moving because of gelation was regarded as the phase transition temperature. Each sample was measured at least in triplicate.

Effect of Dilution on phase transition temperature

The measurements were made at 15-37°C, the temperature in the conjunctival sac of the eye. The sol-gel transition temperature of poloxamer was determined from shearing stress measurements at 500 rpm, the temperature was increased 4°C every 10 min. The GT was defined as the point where a sudden shift in shearing stress was observed. To mimic the properties in the eye, if all applied polymer solution (40 µl) was immediately mixed with the available tear fluid (7 µl), which would be the worst case scenario, the polymer solution was mixed with simulated tear fluid in a ratio of 40:7

In vitro diffusion studies of ciprofloxacin through a membrane

The in vitro diffusion of the drug through a membrane was carried out in a system composed of a glass tube in which a cellophane membrane (HIMEDIA LA 393-1MT) (soaked over night in artificial tear fluid, pH 7.4) was stretched and securely fastened with a rubber band; 1 g of the 0.3% w/w formulation was placed in the tube (phase I or the donor phase). This was hung vertically in a beaker containing 50 ml artificial tear solution, pH 7.4 (phase II or the acceptor phase). The diffusion system was placed in a thermostatically shaking water bath at at 37 ± 1 °C At predetermined time intervals, 1 ml of the solutions were removed from the acceptor phase and analyzed for ciprofloxacin using a UV spectrophotometer (Shimadzu UV-1700(E), 230VCE) at 270 nm and an equal volume of fresh, pre-warmed artificial tear was replaced into the dissolution vessels. The amount of ciprofloxacin released at each time was calculated from a calibration curve.

Antimicrobial efficiency of controlled release ciprofloxacin gel

The antimicrobial efficiency and prolonged effect of selected controlled release ciprofloxacin gel were determined on staphylococcus strains as a function of time. The inhibitory effect of ciprofloxacin formulation on the studied microorganisms was evaluated using agar diffusion test. Wells were punched into the nutrient agar previously seeded with test organisms and wells were filled with 100 µl of the samples. After allowing diffusion of solution for two hour the plates were incubated for 24 hr at 37°C and the diameters of inhibition zones were measured. The inhibitory effect of optimized gel formulation was compared with marketed ciprofloxacin eye drops.

RESULTS AND DISCUSSION

Formulation of gelling solution

Vehicle used and method of preparation has a major contribution in formulation and stability of ciprofloxacin HCl gelling solution. Method of preparation of gelling solution differs with change in polymer used depending upon the physiochemical properties of polymers in addition and as mentioned earlier in introduction, chitosan needs to be dissolved in acetic acid solution which will be used as a vehicle for Poloxamer solution results in clear and transparent gelling solution.

Evaluation studies of ophthalmic gel formulations

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

The appearance, clarity, pH, and drug content of all the gelling solutions were shown in table 2. While optimizing the formulation parameters that primarily in consideration was phase transition temperature, pH, and physical appearance. Poloxamer (CFXF1) in addition with HPMC (CFXF2), chitosan (CFXF3) and PVA (CFXF5 and CFXF6) does not shows any change in appearance. All the formulations excluding CFXF4 show the clear and transparent appearance. Addition of PVP (CFXF4) to the poloxamer-chitosan polymeric solution changes color to light yellow. All the formulations shows percentage drug content within the range of 89.16% to 98.54%. In the process of optimization pH of all the formulation was adjusted to 7.4 to avoid ocular irritancy.

Table 2: Evaluation parameters of ophthalmic gel formulations

Formulations Tests	CFXF1	CFXF2	CFXF3	CFXF4	CFXF5	CFXF6
Physical Appearance	Transparent	Transparent	Transparent	Light yellow	Transparent	Transparent
Clarity	clear	Clear	Clear	cloudy	clear	clear
pH	7.4	7.4	7.4	7.4	7.4	7.4
Gelation capacity	++	+++	++	+	+++	+++
Drug Content (%)	89.16	91.58	98.54	98.17	98.35	98.35

(+: Gels slowly and dissolves, ++: Gelation immediate and remains for a few hours, +++: Gelation immediate and remains for an extended period)

In vitro release studies of ciprofloxacin HCl through a membrane

The cumulative percentage of ciprofloxacin released from the formulation as a function of time is shown in Fig. 1. Ciprofloxacin release from optimized formulation was highly depends upon the concentration of polymer used. In due reference, drug release of poloxamer (CFXF1) was studied over combination of poloxamer-HPMC (CFXF2) and the result shows that percentage drug release from combination poloxamer- HPMC (CFXF2) was much lower (14.33%) than poloxamer alone(19.65%) (CFXF1). This might be due to the hydrophilic nature of HPMC and ciprofloxacin HCl. A study shows that active substance release from the optimized formulation with fixed poloxamer concentration increases as the chitosan concentration increases from 0.1-0.2 %w/w. For the formulation containing poloxamer only (CFXF1) with no chitosan shows 9.54% of cumulative release after 4 hour and19.65% after 8 hour .when chitosan was incorporated with poloxamer at the concentration of 0.2%w/w cumulative percentage release was 22.52 % in 4 hour and 31.86% after 8 hour. The increase in concentration of chitosan from 0.25w/w or above failed in optimization of formulation due to failure in phase transition temperature at temperature 35-37°c. cumulative percentage release was found to increase from 31.86% to 42.92% when PVA(0.2%w/w) was added to poloxamer chitosan polymeric mixture(CFXF5). In next formulation an attempt was made to increase the percentage drug release by increase PVA concentration to 0.3%w/w (CFXF6) and results in further increase in percentage drug release up to 49.61%. This increase in percentage drug release with increase in PVA concentration shows flux enhancing action in gel formulation.

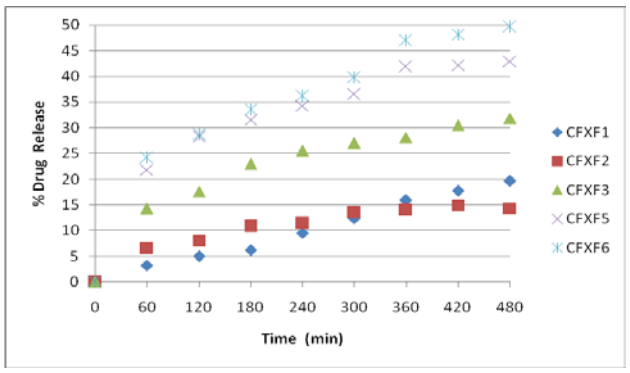

Fig. 1: In-vitro drug release from ophthalmic gel

Phase Change Temperature and Effect of Dilution On Phase Change Temperature

Phase transition temperature and effect of dilution on phase transition temperature of all formulation shows in table 3. Concentration of Poloxamer used in ophthalmic formulation was in the range of 18 – 24 % w/w and within that range it was found that increase in concentration decreases the phase transition temperature. Formulation (CFXF1) 20 % w/w concentration shows phase transition temperature of 38.5°c. Addition of PVA in range of 0.2-0.3%w/w does not show any measurable change in phase transition temperature of formulations. A pre-formulation study shows that increase in chitosan concentration above 0.2%w/w in combination with 20%w/w poloxamer does not show any phase transition. Addition of PVP to Poloxamer (CFXF4) fails for gelation.

Table 3: Comparison of Phase Change Temperature (PCT) Of *In Situ* Ophthalmic Gels of Ciprofloxacin before and After Dilution with Simulated Tear Fluid. (Mean ±SD)

Formulation Code	PCT Before Dilution (°C)	PCT After Dilution (°C)
CFXF1	38.5 ± 1.2	39.5 ± 0.1
CFXF2	28.0 ± 0.0	33.0 ± 0.0
CFXF3	39.8 ± 0.2	41.8 ± 1.0
CFXF4	37.1 ± 0.1	39.1 ± 0.5
CFXF5	37.5 ± 1.8	38.6 ± 0.6
CFXF6	37.1 ± 0.2	38.5 ± 0.2

Antimicrobial Efficiency of Controlled Ciprofloxacin HCl Gel Formulations:

The result of the antimicrobial efficacy tests shown in table 4. Ciprofloxacin gel formulation and ciprofloxacin marketed eye drops shows measurable difference in area of zone of inhibition (fig.2). The higher ZOI values obtained for the formulations in comparison to the standard could be attributed to the slow and prolonged diffusion of the drug from the polymeric solution due to its higher viscosity. The study indicates that ciprofloxacin retained its antimicrobial activity after formulated into gelling solution.

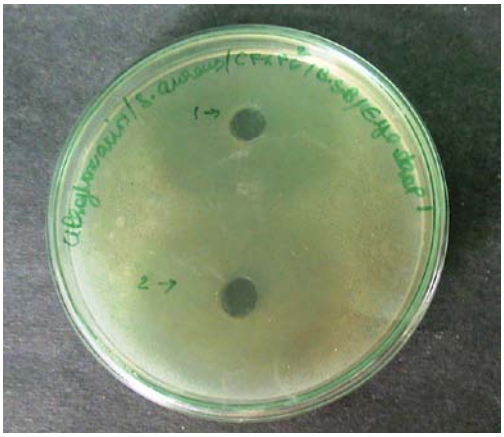

Fig. 2: Comparative Zone of inhibition between ciprofloxacin formulated gel (2) and marketed eye drops (1)

Table 4: zone of inhibition (ZOI) for Staphylococcus aureus

Formulations	Zone of Inhibition (mm) Mean ±SD
Marketed eye drop	31.5 ± 1.1
CPXF6	34.4 ± 0.1

CONCLUSION

Poloxamer alone responsible for Thermoreversible phase transition. Depending upon the concentration used phase transition temperature differs. Increase in poloxamer concentration shows decrease in phase transition temperature within the range of 18-24%w/w. Addition of HPMC decrease percentage drug release.PVP with poloxamer fails in optimization, as fail to give phase transition.PVA does not show have any effect on phase transition but increase drug release efficiency. Result of this study indicate that the

formulation with polymer concentration at poloxamer 407 at 20 % w/w, chitosan 0.2%w/wand PVA 0.3% w/w, will be expected to give highest drug release efficiency with acceptable phase transition temperature and delivers drug for 8-hour.

ACKNOWLEDGEMENT

BASF (Ludwigshafen, Germany) for gift sample of Poloxamer P407, Inventia healthcare for gift sample of Ciprofloxacin and Dr. A. M. Ittadwar, Principal, Gurunanak College of Pharmacy, Nagpur for providing facilities for the successful completion of research work.

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