International Journal of Pharma and Bio Sciences V1(2)2010

STUDY OF A NOVEL ENVIRONMENTALLY RESPONSIVE OPHTHALMIC DRUG DELIVERY SYSTEM

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ABSTRACT

Targeting the drug to the appropriate site of action is usually one of the greatest challenges in drug delivery to the eye because of its anatomical and physiological nature. Pre-application an environmentally responsive systems are in the liquid state and easily administered in to eye whereas post-application, they are transformed in highly viscous gel. In present study temperature triggered and ion activated environmentally responsive system was prepared for ocular drug delivery by using poloxamer-407 and sodium alginate. All prepared formulations were transparent and clear and the gel formed in vitro produced sustained drug release up to eight hours. Rheological behaviors of all formulations were not affected by addition of model drug. Antimicrobial activity of drug was not changed significantly due to formulation ingredients and conditions as compared to reference formulation (Marketed formulation). As the concept involved is novel and the methodology used for the preparation is simple as that of conventional ophthalmic liquid dosage form, it is industrially oriented and economical.

KEYWORDS

Environmentally responsive, Temperature triggered, Poloxamer, Sodium alginate.

INTRODUCTION

Topical administration of drugs to the eye is the most common method for treatment and diagnosis of ocular diseases. But this route show low bioavailability of drugs because of low residence volume (7-10 µl) permanently, loss of administered dose due to rapid clearance lachrymation, non-productive absorption through conjunctiva and naso- lachrymal drainage[1].

Several new preparations have been developed for ophthalmic use, not only to prolong the contact time of the vehicle on the ocular surface but also to slow down drug elimination. Successful results

STUDY OF A NOVEL ENVIRONMENTALLY RESPONSIVE OPHTHALMIC DRUG DELIVERY SYSTEM

have been obtained with inserts and collagen shields. However, these preparations have some disadvantages such as poor compliance, especially by elderly people and many patients sometimes lose the device without noticing it[2].

Various types of dosage forms including liquid drops, gels, ointments and solid ocular inserts (both degradable and nondegradable) are used for delivery of therapeutic agents[3-5]. Another interesting delivery platform is environmentally responsive polymer that undergoes a phase transition after application[6].A more desirable dosage form would be one that can be delivered in a drop form, creates little to no problem for vision, and needs to be dosed no more frequently than once or twice daily. Several types of polymers have been utilized to this end. *In situ* activated gel-forming systems can be described as viscous liquids that upon exposure to physiological conditions will shift to a gel phase.

The principal advantage of this formulation is the possibility of administering accurate and reproducible quantities, in contrast to already gelled formulations and moreover promoting precorneal retention. This new concept of producing a gel *in situ* was suggested for the first time in the early 1980s[2]. Significant increase in the pre-corneal residence time of drugs and consequently bioavailability can be achieved by using delivery systems based on the concept of environmentally responsive system (*In situ* gel-forming systems).

Environmentally responsive system have been produced that exhibit dramatic changes in their swelling behavior, network structure, permeability and mechanical strength in response to a number of external stimuli, including pH, ionic strength of the surrounding fluid, temperature, presence of specific solutes and applied electrical or magnetic fields[7].Ion-activated environmentally responsive ophthalmic system (0.3% (w/v)) using alginate (Kelton®) as a gelling agent in combination with HPMC E50Lv as a viscosity-enhancing agent was successfully formulated. The formulation underwent gelation in the cul-de-sac upon instillation as drops into the eye. The gel formed in vitro produced sustained drug release over an 8-h period[8].Poloxamer 407 (trade name, Pluronic F-127), a non-toxic poly(ethylene oxide)/poly(propylene oxide)/ poly(ethylene oxide triblock copolymer with a weight-average molecular weight of 12,600, contains 70% hydrophilic ethylene oxide units and 30% hydrophobic propylene oxide units. It forms a gel on warming to body temperature by undergoing a sol–gel transition. As a result of this reverse thermal gelation and extremely low toxicity, the administered solution containing drug turns into a gel and renders slow release characteristics to the drug delivery system in the pharmaceutical fields[9-11].

The objective of present study was to develop a temperature triggered and ion activated environmentally responsive system for ofloxacin. It is a fluoroquinolone derivative used to treat external infections of eye such as acute and subacute bacterial conjunctivitis, keratitis, keratoconjuctivitis and corneal ulcers, which can prevent frequent drug administration and enhance patient compliance. Sodium alginate was used as ion activated gelling agent in combination with Poloxamer 407 as temperature triggered gelling agent for formulation of ofloxacin eye drops (0.3% w/v), which undergo gelation in simulated tear fluid and provide sustained release of drug.

STUDY OF A NOVEL ENVIRONMENTALLY RESPONSIVE OPHTHALMIC DRUG DELIVERY SYSTEM

MATERIALS AND METHODS

Ofloxacin hydrochloride was obtained as a gift sample from Cadila Health Care Ltd., Ahmedabad, India. Sodium alginate was purchased from Loba chemie, Mumbai, India. Poloxamer-407 was kindly gifted by BASF Ltd., Mumbai., India. All other reagents and chemicals used were of analytical grade.

Preparation of novel environmentally responsive ophthalmic drug delivery system

The formulations were prepared using 0.3%w/v of Ofloxacin, benzalkonium chloride (antimicrobial preservative), different grades of methocel and sodium alginate in different concentration. All glass wares were soaked in hot cleaning solution, rinsed with distilled water, drained and placed in dust free environment. They were sterilized in hot air oven at 160°C for 1 hr. Closures were cleaned by washing with a detergent and rinsing with purified water and placed in benzalkonium chloride solution (0.02%w/v) and were subjected to saturated steam at 115°C to 116°C for 30 min. Solution 1 was prepared by dispersing appropriate quantities of Sodium alginate in 25 ml of citrophosphate buffer (pH 6). Solution 2 was prepared by dispersing required quantities of Poloxamer-407 in 50 ml cold citrophosphate buffer. In case of solution 3, ofloxacin was dissolved in 1 ml of glacial acetic acid followed by addition of distilled water and benzalkonium chloride. Solution 1 and 2 were mixed together, after uniform mixing solution 3 was added and final volume was made by adding remaining quantity of Citrophosphate buffer (Tab. 1). Finally, the resultant solution was subjected to membrane filtration by using cellulose nitrate membrane of pore size of 0.22µm.

Table 1
Formulation of novel ophthalmic drug delivery system

Ingredients	SP1	SP2	SP3	SP4
Ofloxacin (g)	0.3	0.3	0.3	0.3
Sodium alginate (g)	0.4	0.4	0.4	0.4
Poloxamer-407	15	16	17	18
Benzalkonium chloride (g)	0.02	0.02	0.02	0.02
Distilled water	q.s.	q.s.	q.s.	q.s.
Citrophosphate Buffer (ml) q.s.	100	100	100	100

Characterization of drug and polymer

STUDY OF A NOVEL ENVIRONMENTALLY RESPONSIVE OPHTHALMIC DRUG DELIVERY SYSTEM

Drug and polymer compatibility study

Physical mixtures (1:1 combination) of drug and polymer were studied first for there compatibility. The interaction between the drug and polymer was studied by using the transmission Fourier transform infrared (FTIR) spectrometry (IR affinity I, Shimadzu Corporation, Japan). An infrared spectrum of ofloxacin and selected polymer was taken individually then there physical mixtures were prepared and stored at accelerated conditions. The resultant IR spectrum of physical mixture was compared with individual drug and polymer spectrum for any signs of incompatibility.

In vitro evaluation of formulations

Physical tests of novel ophthalmic solution

Test for appearance/ clarity

All the formulations were checked for general appearance i.e. color, odour, any suspended particulate matter etc. The clarity was checked using wooden board with black and white background. The vials were held horizontally and gently rotated immediately under the lamp and then inverted once or twice to detect foreign particles.

Determination of pH

The pH of each formulation was recorded using a digital pH meter. The pH meter was calibrated before each use with buffer solutions of pH 4 and pH 7.The pH of all formulations was recorded immediately after preparation as well as after 24 hours of storage at room temperature.

Gelation Studies

The gelling capacity was determined by placing 100 µl of prepared system in a vial containing 2 ml of artificial tear fluid freshly prepared and equilibrated at 35 °C. The gel formation was visually evaluated; time for gelation and the time taken for the gel to dissolve were noted. The composition of artificial tear fluid was sodium chloride (0.670 g), sodium bicarbonate (0.200 g), calcium chloride·$2H_2O$ (0.008 g), and purified water q.s. (100 g)[12].

The lowest scores (+) were assigned to those products in which the phase transition occurred only after 60-90 sec. and the formed gels collapsed within 1-2 hrs. The highest scores (+++) were assigned to those products for which the phase transition commenced within 60-90 sec. and the gels so formed were stable for about 7-8 hrs. The moderate scores (++) were assigned to the products, which could form the gel in 60-90 sec. but failed to maintain gel structure for more than 3 hrs.

Test for performance characteristics of novel ophthalmic solutions

Drug content

The drug content was determined by taking 1ml of the formulation and diluting it to 100 ml with distilled water. Aliquot of 5ml was withdrawn and further diluted to 25 ml with distilled water. Ofloxacin concentration was determined at 287 nm by using UV-Visible spectrophotometer (UV-1200, Shimadzu Corporation, Japan).

Rheological studies

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 Brookefield LVDV-II+ Pro viscometer with angular velocity run from 1 to 100 rpm.

In vitro release studies

STUDY OF A NOVEL ENVIRONMENTALLY RESPONSIVE OPHTHALMIC DRUG DELIVERY SYSTEM

The in vitro release of ofloxacin was studied from the all formulations through a cellophane membrane, using a modified USP XXIII dissolution test apparatus. The dissolution medium was freshly prepared artificial tear fluid (pH 7.4). A cellophane membrane was tied to one end of fabricated glass cylinder (5 cm inner diameter, closed at one end). A 2-ml volume of the formulation was accurately pipetted into to this apparatus and it was attached to metallic driveshaft of dissolution test apparatus (Electrolab, 8-ST) and immersed in 50 ml of dissolution medium maintained at 37±1 ^{0}C with a rotating speed of 50 rpm. Samples, each 1 ml in volume, were withdrawn at hourly intervals and replaced by an equal volume of receptor medium. The release of ofloxacin was analyzed by UV spectrophotometry at 287 nm[13].

Antimicrobial efficacy studies

Antimicrobial efficacy test was carried out by cylinder plate method. 1000 µl of marketed solution of ofloxacin (standard solution) and developed formulation diluted with citrophosphate buffer, pH 6.0 (test solution) were poured in to cups (3 mm diameter) bored in to sterile nutrient agar. It was previously seeded with *Staphylococcus aureus* (NCIM 2079). After allowing diffusion of the solutions for 2h, the agar plates were incubated at 37 ^{0}C for 24h. The zone of inhibition (ZOI) measured around each cup was compared with Standard solution.

Accelerated stability studies

Selected formulation (SP4) was stored at 40°C and 75% RH and 25°C and 60%RH for a period of 3 months. The formulation was evaluated at periodic intervals for drug content, clarity, pH, sol–gel transition, rheology, in vitro drug release and sterility.

RESULTS

Drug and polymer compatibility study

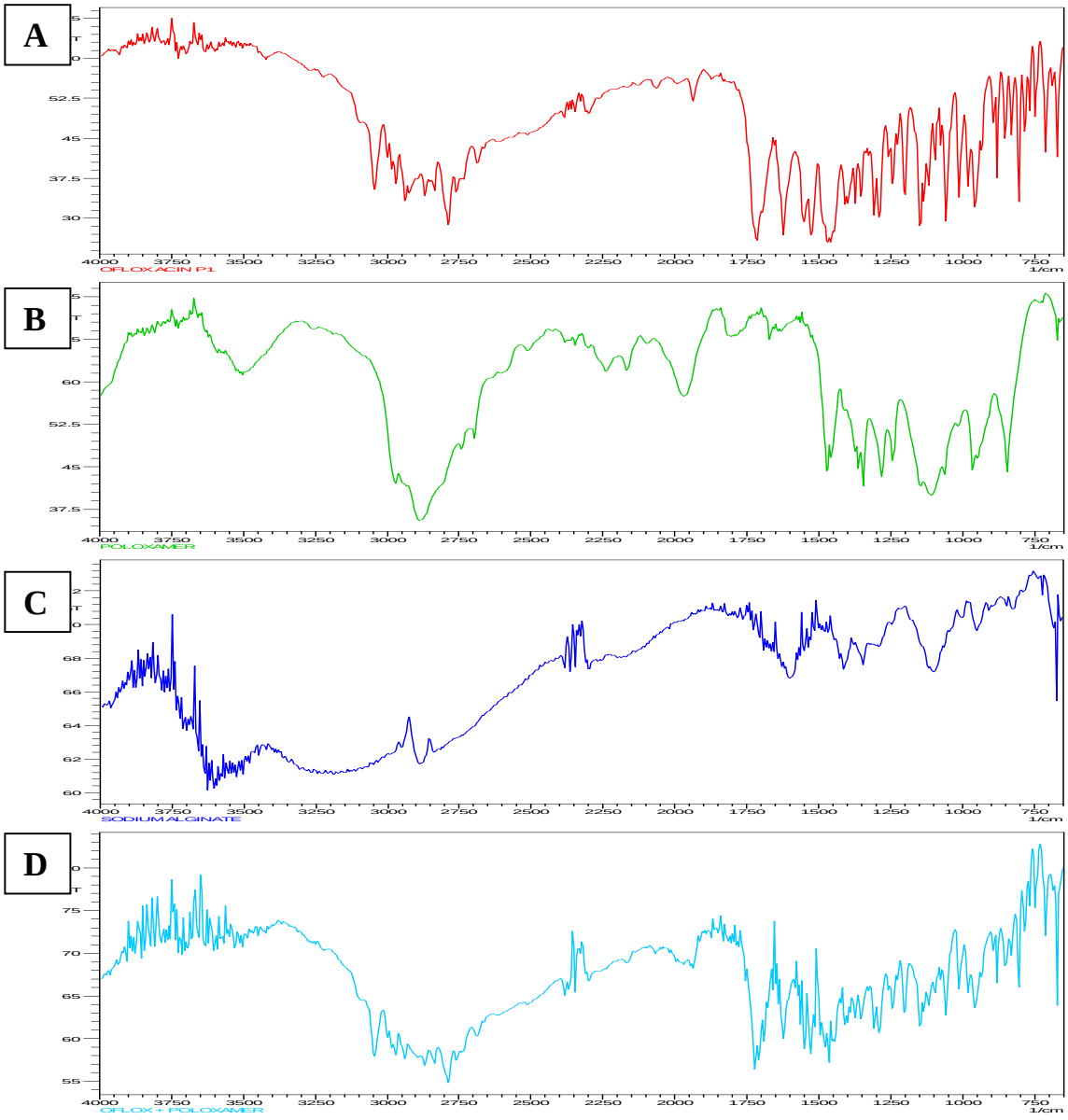
As shown in Fig. 1, there was no significant difference in the FTIR spectra of physical mixtures of drug and polymers when compared to the spectra of individual components.

In case of ofloxacin, the principal peaks are at wave number 1459, 1621, 1715, 1086 cm^{-1}. Generally, the carboxylic acid OH band will be in the region of 3300 – 2500 cm^{-1}. This is usually a weak band indicating that hydrogen bonding with the carbonyl group is present. The peak at 1715 cm^{-1} shows the carbonyl stretching (C=O) of carboxylic acid

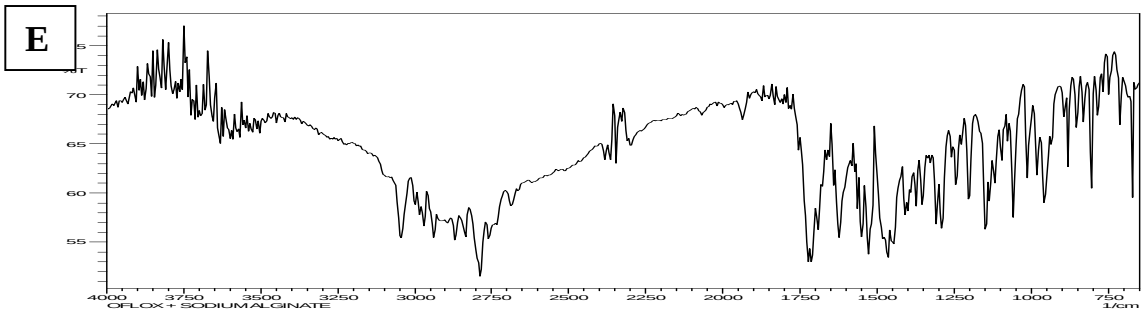
Figure 1.

STUDY OF A NOVEL ENVIRONMENTALLY RESPONSIVE OPHTHALMIC DRUG DELIVERY SYSTEM

FTIR Spectra of drug and polymers



STUDY OF A NOVEL ENVIRONMENTALLY RESPONSIVE OPHTHALMIC DRUG DELIVERY SYSTEM



A – Pure ofloxacin *B – Pure poloxamer-407* *C – Pure sodium alginate*
D – ofloxacin + poloxamer-407 *G – ofloxacin + sodium alginate*

Test for appearance/ clarity

When all formulations were observed against black and white background for clarity, all formulations were clear and transparent in appearance, as shown in table 2.

Table 2
Data for appearance/ clarity of ophthalmic formulation

S.No.	Formulation Code	Observation
1	SP1	Transparent
2	SP2	Transparent
3	SP3	Transparent
4	SP4	Transparent

Determination of pH

The pH of all the formulations was found in the range of 5.9 to 6.5. Ideally, the ophthalmic solutions should have possessed the pH in the range of 4.5-11.0.

Table 3
Observed pH ranges for sterile ophthalmic formulations

S.No.	Formulation Code	Observed pH range
1	SP1	6.1
2	SP2	5.9
3	SP3	6.2
4	SP4	6.5

STUDY OF A NOVEL ENVIRONMENTALLY RESPONSIVE OPHTHALMIC DRUG DELIVERY SYSTEM

Gelation Studies

The numerical scores for gelling capacity were assigned to experimental formulations depending upon time required for phase change and uniformity and viscosity of formed gel and time taken to dissolve the gel. Table 4 shows the comparative scores for all the formulations**.**

Table 4
Gelling capacity of ophthalmic formulations in STF(Simulated tear fluid)

S.No.	Formulation Code	Gelling capacity
1	SP1	+
2	SP2	++
3	SP3	+++
4	SP4	+++

+ gelation in 60-90 sec. and the formed gels collapsed within 1hr; ++ gelation in 60-90 sec. but failed to maintain gel structure for more than 3 hr; +++ gelation in 60-90 sec. and stable for about 7-8 hr.

Drug content

Table 5, shows ofloxacin concentration in formulations, it was determined at 287 nm by using UV-Visible spectrophotometer. The absorbance of solution at 287 nm was used to calculate percentage drug content. Drug content of ofloxacin hydrochloride in all 9 formulations was between 98.61% - 102.3 %.

Table 5
Drug contents of ophthalmic formulations

S.No.	Formulation Code	Average % Drug content
1	SP1	102.3 ± 0.73
2	SP2	98.61 ± 2.62
3	SP3	98.43 ± 1.42
4	SP4	98.79 ± 1.07

± SD (n=3)

Rheological studies

The viscosity of liquid ophthalmic formulation is generally in range of 3-40 cps. This is needed for two reasons, (a) Proper instillation of drops from containers, (b) Good spreadability of solutions within the eye. The viscosity of the formulations SP1 to SP4 ranged from 12-20 cps.

STUDY OF A NOVEL ENVIRONMENTALLY RESPONSIVE OPHTHALMIC DRUG DELIVERY SYSTEM

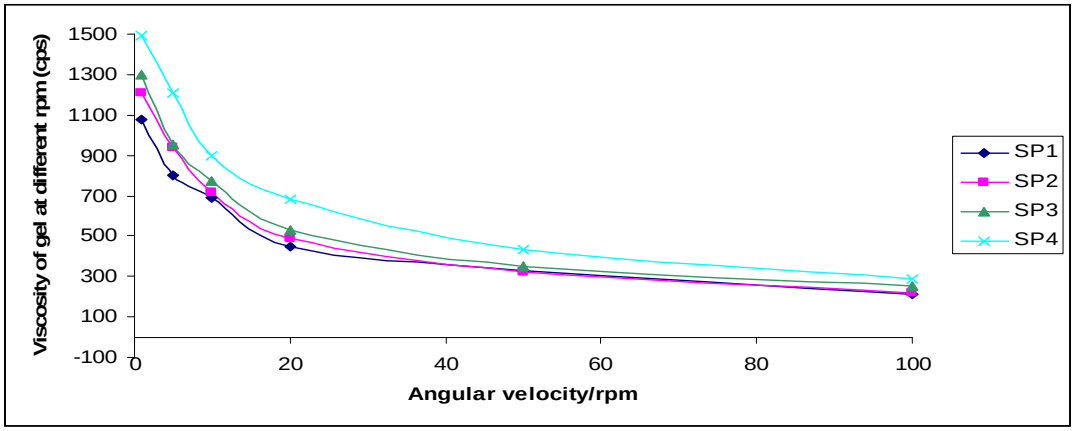
Table 6
Viscosity of ophthalmic formulation (solution)

S.No.	Formulation Code	Viscosity (cps)
1	SP1	12.0
2	SP2	15.0
3	SP3	17.0
4	SP4	20.0

Table 7
Viscosity values for ophthalmic solutions after phase transition (gel) in the simulated tear fluid (STF).

S. No.	Formulation Code	Viscosity of gel at different RPM (cps)					
		1	5	10	20	50	100
1	SP1	1080	800	690	450	330	210
2	SP2	1210	940	720	490	320	220
3	SP3	1300	950	770	530	350	250
4	SP4	1490	1210	900	680	430	290

Figure 2
Viscosity values for ophthalmic solutions after phase transition (gel) in the simulated tear fluid (STF).



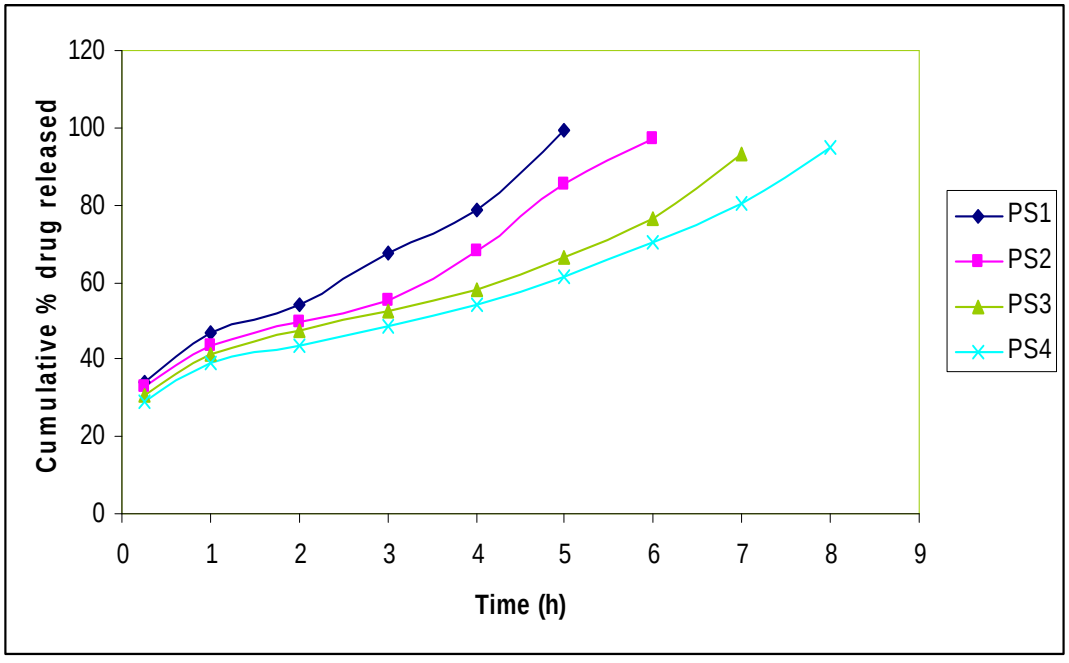
STUDY OF A NOVEL ENVIRONMENTALLY RESPONSIVE OPHTHALMIC DRUG DELIVERY SYSTEM

In vitro release studies

Fig. 3 shows the cumulative amount of ofloxacin released versus time profiles for different drug-containing solutions. Formulation S1 released almost all drug within 5 hrs. For SP4 highest concentration of poloxame-407 was used and the drug released was sustained up to 8 hrs.

Figure 3
Cumulative amount of ofloxacin released from nine different formulations.

Antimicrobial efficacy studies

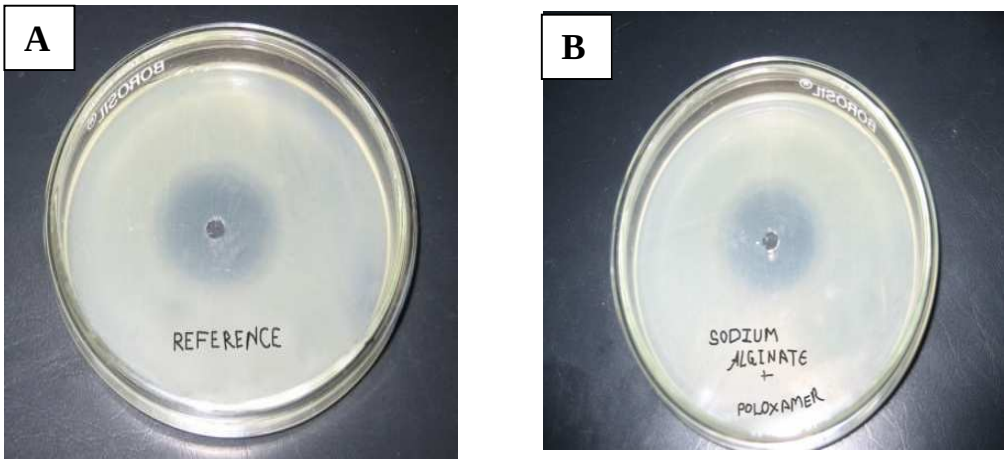


The following photographs also support the findings of antimicrobial testing of Ofloxacin in standard and formulation containing polymer.

STUDY OF A NOVEL ENVIRONMENTALLY RESPONSIVE OPHTHALMIC DRUG DELIVERY SYSTEM

Figure 4

Photographs of antimicrobial efficacy test A. marketed solution of ofloxacin (standard solution) and B. developed formulation (SP4)



Accelerated stability studies

The formulation SP4 was stored at different storage conditions. The conditions were 40°C and 75% RH and 25°C and 60% RH for a period of three months. The results for drug content, clarity, pH, sol–gel transition, rheology, in vitro drug release and sterility were studied.

DISCUSSION

Some researchers have reported environmentally responsive systems made up of either sodium alginate or its combination with different polymers (ion activated system). Some have investigated the systems prepared from poloxamer-407 (thermosensitive system) but problem with this system is the concentration of poloxamer-407 which is generally very high. Present investigation focused on combination of these two polymers which work on totally different mechanisms.Prior to formulate the system, drug and polymers were subjected for compatibility study, In this study, the interaction between the drug and polymer was studied by using the transmission Fourier transform infrared (FTIR) spectrometry. As shown in fig. 1 there was no change in major peaks, it can be concluded that there was no significant interaction between drug and polymer.

Prepared formulations were assessed for appearance and clarity test, on careful visual inspection against dark and white background; all the solutions were found to be clear and

STUDY OF A NOVEL ENVIRONMENTALLY RESPONSIVE OPHTHALMIC DRUG DELIVERY SYSTEM

transparent. The pH of all the formulations was found in the range of 5.9 to 6.5, which it may minimize any discomfort after administration. Ideally, the ophthalmic solutions should have possessed the pH in the range of 4.5-11.0, so as to minimize discomfort and irritation to the eye.

Results from in vitro gelation studies suggest that, formulations SP3 and SP4 has good gelling capacity, phase transition is a major precondition for environmentally responsive system. The gel structure was stable for 7-8 hours, it suggest that it can prolong the therapeutic effect of drug by increasing corneal contact time. The drug content of all formulation was in acceptable limit; this is the primary indication of stability of formulated products and indicates that addition of polymer not affect the drug content of formulation.Figure 2 reveals that all formulations follow pseudoplastic flow characteristic, it can be assume that it will not disturb the normal blinking mechanism of eye. From the data obtained from rheological studies it was observed that all formulations had less viscosity at room temperature but it becomes highly viscous rheologically structured network as it comes in contact with STF. It may be due to ionic cross liking of cations present in STF and alginic acid chains as well as due to thermosensitive nature of poloxamer-407. The rheological behaviors of all formulations were not affected by addition of ofloxacin. Since the ocular shear rate is very high, ranging from 0.03 s^{-1} during inter-blinking periods to 4250-28500 s^{-1} during blinking, viscoelastic fluids with a viscosity that is high under low shear rate conditions and low under the high shear rate conditions are often favored.Figure 3 shows the cumulative amount of ofloxacin released versus time profiles for different drug-containing solutions. All the solutions contained 0.3% (w/v) ofloxacin. All formulations show initial burst release due to aqueous vehicle, but as the diffusion study goes on there was an increase in the viscosity of the formulation and drug release was retarded because the release of drug from gel is inversely proportional to the gel strength. In case of formulation SP4, 31% of drug was released within one hour but the release rate was gradually retarded up to 94% after eight hours. Formulation SP4 has a better ability to retain drugs than other polymer solutions (formulation PS1, PS2 and PS3). The results of the antimicrobial efficacy tests in form of photographs are shown in figure 4. Antimicrobial study shown that, there was no significant change in the antimicrobial activity of drug due to formulation ingredients and conditions as compared to reference formulation (Marketed formulation). The zone of inhibition of reference solution was 33 mm, whereas zone of inhibition of test solution was 30 mm. It indicates that antimicrobial efficacy of formulation SP4 was 90.90%, as compared with reference solution.Accelerated stability study was carried out for three months. The results for drug content, clarity, pH, sol–gel transition, rheology, in vitro drug release and sterility at accelerated conditions were satisfactory. Hence it can be concluded that there was not significant interaction between the components of formulation.As the concept involved is novel and the methodology used for the preparation is simple as that of conventional ophthalmic liquid dosage form, the developed system is industrially oriented and economical alternative to conventional ophthalmic formulations.

STUDY OF A NOVEL ENVIRONMENTALLY RESPONSIVE OPHTHALMIC DRUG DELIVERY SYSTEM

ACKNOWLEDGEMENT

The authors are grateful to Prof. S. J. Surana and Dr. S. G. Gattani (R.C.P.I.P.E.R., Shirpur, India) for providing necessary facilities to carry out research work

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