

**ENVIRONMENTALLY RESPONSIVE OPHTHALMIC GEL FORMULATION: BASED ON COMBINATION WITH
NATURAL POLYCATIONIC COPOLYMER**

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ABSTRACT

The purpose of the present investigation was to prepare and evaluate environmentally responsive formulations for improved drug residence time with corneal epithelium. The formulations so prepared were in the liquid state at 4°C while turned into a gel at the temperature of the Cul-de-sac. Poloxamer 407 was used as the polymer which exhibited the phase transition behavior. Natural polycationic polymer chitosan was used to improve residence time. The prepared formulations were characterized for physical appearance, content uniformity, in-vitro drug diffusion, pH, gel strength, viscosity and gelation temperature. The formulations exhibited drastic increase in the viscosity at the temperature of 37°C indicating their possible use as in-situ gelling systems. Out of all the formulations studied the poloxamer chitosan PVA formulation Shows the desired drug with prolond contact time.. Ciprofloxacin Hydrochloride was used as model drug.

Keywords: chitosan, thermoreversible, cul-de-sac, etc.

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INTRODUCTION

Although various formulation exists in market for ocular drug delivery but are not able to provide highest bioavailability related to administered dose^[1]. Whenever an ophthalmic drug is applied through an conventional dosage form to the anterior segment of the eye, only small amount

(5%) actually penetrates the cornea and reaches the interior tissue of the eyes^[2-3] Factors that affects drug bioavailability includes rapid solution drainage by Gravity, Induced lachrymation, Blinking reflex, Normal tear turnover, Superficial absorption of drug into the conjunctiva and sclera and rapid removal by the peripheral blood flow, Low corneal

permeability (act as lipid barrier). The progress has been made in gel technology for the development of droppable gel. They are liquid upon instillation and undergo phase transition in the ocular cul-de-sac to form visco-elastic gel and this provides a response to environmental changes. Thus objective of this study was to develop an ophthalmic system that shows prolonged contact time with corneal epithelium, Simplicity and installation for patient, Non-irritable and comfortable form and with appropriate rheological considerations [3]. Poloxamer 407 with its thermoreversible gelation and surface active properties was utilized to formulate environmentally controlled in situ gel delivery system for ocular use. Chitosan is a natural polycationic copolymer consisting of glucosamine and N-acetylglucosamine units [26]. The polymer has valuable properties as a biomaterial because it is considered to be biocompatible, biodegradable and non-toxic. The positively charged polysaccharide chitosan is able to increase precorneal residence time of ophthalmic formulations containing active compounds when compared with simple aqueous solutions. Ciprofloxacin HCl (1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid hydrochloride) was used as model drug. It shows its Pharmacological action by Inhibition of DNA gyrase (Topoisomerase II) which mediate the formation of supercoils of DNA

Materials and Methods

Poloxamers P407 was obtained as a gift sample from BASF Corp. (Ludwigshafen, Germany);

Ciprofloxacin HCl was kindly gifted from Inventia healthcare Pvt. Ltd. (INDIA). Chitosan and PVA were purchased from MERK. Triethanolamine, Benzalkonium chloride was obtained from Research lab fine chem. industries (INDIA). All other chemicals used were of analytical grade.

Formulation Of Poloxamer-Chitosan Ophthalmic Gel

The formulations were prepared on a weight/weight basis using the cold method. Appropriate amounts of P407 was added to bidistilled water (4°C). The dispersions were stored in a refrigerator at 4°C over night results in clear solution. Chitosan was initially dissolved in a solution of acetic acid (0.5% v/v) and used as a solvent for the poloxamer dispersion was then kept in a refrigerator at 4°C over night results in clear solution. To the above solution PVP and HPC was dissolved to obtain desired polymeric solutions. PVA was dissolved in bidistilled water, stirred to make clear colorless solution and added to chitosan - Poloxamer polymeric solution. For preparation of ciprofloxacin-containing polymer solutions, 0.3% of ciprofloxacin was added to the Polymeric solutions with continuous stirring until thoroughly mixed. Benzalkonium chloride (0.01%) and NaCl (q.s. 0.9%) were added as preservative and tonicity modifier respectively in all solutions. All the sample solutions were adjusted to pH 7.4 by triethanolamine, sterilized at 121°C and 15 psi for 20 min and then stored in the refrigerator prior to further evaluation.

Table 1: Formulation of Poloxamer-Chitosan-PVA Ophthalmic Gel Characterization of Ciprofloxacin Gel

Formula Variation(%w/w)	CPXF1	CPXF2	CPXF3	CPXF4	CPXF5	CPXF6	CPXF7
Ciprofloxacin HCl	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Poloxamer	20	20	20	20	20	20	20
Chitosan	---	0.20	0.20	0.20	0.20	0.20	0.20
PVP	---	---	0.20	---	---	---	---
HPC	---	---	---	0.20	---	---	---

PVA	---	---	---	---	0.20	0.30	0.40
NaCl	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Triethanolamine	qs	qs	qs	qs	qs	qs	qs
Benzalkonium Chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Purified water	q.s. 100	q.s. 100	q.s. 100	q.s. 100	q.s. 100	q.s. 100	q.s. 100

pH ^[4].

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 4.0 and 7.0 In the process of optimization pH of all the formulation was adjusted to 7.4 to avoid ocular irritancy.

Determination Of Visual Appearance And clarity ^[5].

The appearance and clarity were determined visual under fluorescent light against black and white background in well lit cabinet.

Isotonicity Evaluation ^[6].

Different Formulations were mixed with few drops of blood and observed under Motic digital microscope at 40X magnification and compared with standard marketed ophthalmic formulation containing ciprofloxacin.

Uniformity Of Drug Content ^[7].

Drug content of ciprofloxacin formulations was determined by dissolving 100 mg of formulation 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 274.2 nm.

Measurement Of Phase Change Temperature ^[8].

An aliquot of 2 mL of formulation was transferred to a test tube and sealed with a parafilm. The tube was maintained in a water bath at 4°C. The temperature of the water bath was increased gradually in increments of 3°C in the beginning of the experiment and then 1°C increments in the region of sol–gel transition temperature (25–34°C) and 0.1°C when it approaches gelation. The tested

formulation was left to equilibrate for 10 min at each new setting. The gelation is considered to be occurred when the meniscus of the formula would no longer move upon tilting through angle 90°.

Rheological Study ^[9-10].

Rheological studies of different gelling solutions were carried out by Brookfield Viscometer LV II +Pro (model-MLVT115) using spindle number S18 at 10 rpm and S96 at 0.3 rpm for gelling solution and gel respectively. Rheology of optimized formulations was carried out at temperature of 4⁰c and elevated temperature (37⁰c).

In Vitro Diffusion Studies Of Ciprofloxacin Through A Membrane ^[4].

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) 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 22.5 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 A-1700)) at 274.2 nm and an equal volume of fresh, pre-warmed artificial tear was replaced into the dissolution vessels.

RESULT AND DISCUSSION:

Evaluation Studies Of Ophthalmic Gel Formulations:

determination of visual appearance, clarity, ph, gelation capacity and drug content:

All the evaluation parameters stated above were shown in table 2. The appearance of all the gelling solutions and gel formed were transparent and clear with few exceptions like CPXF3 and CPXF7. Out of which CPXF7 was unable to form gel. So one may conclude that concentration above 0.3% w/w PVA restricts the gel formation. All the formulations shows percentage drug content within the range of 90.80% to 99.1%. pH of all the formulation were in at 7.1 ± 0.3

isotonicity evaluation

The shape of blood cell was compared with standard marketed ophthalmic formulation containing Ciprofloxacin HCl. The shape and size of blood cell was found to be same or nearly same as that of blood cell with standard marketed formulation (as shown in fig.1 and fig. 2)



Fig 1: marketed formulation

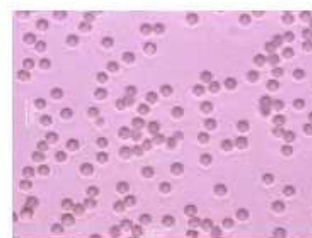


Fig 2: Optimized formulation (CPXF6)

Table 2: Results of Formulation of Poloxamer-Chitosan-PVA Ophthalmic Gel

(Values are Mean \pm SD n=3) (+: Gels slowly and dissolves, ++: Gelation immediate and remains for a few hours, +++: Gelation immediate and remains for an extended period)

Sr No.	Formulation Code	CPXF1	CPXF2	CPXF3	CPXF4
	Evaluation				
1	Clarity Solution	Clear	Clear	Not Clear	Clear
2	Clarity Gel	Clear	Clear	Whitish	Clear
3	Appearance Solution	Transparent	Transparent	Cloudy	Transparent
4	Appearance Gel	Transparent	Transparent	Cloudy	Transparent
5	pH	6.8	7.1	7.4	6.9
6	Gelation Temp. ($^{\circ}$ C)	28.75 ± 0.25	32.30 ± 0.20	41.20 ± 0.10	30.85 ± 0.35
7	Gelation After Dilution $^{\circ}$ C	31.35 ± 0.15	36.35 ± 0.25	32.55 ± 0.15	34.35 ± 0.25
8	Gelation Capacity	++	++	+++	++
9	Drug Content %	90.80 ± 0.77	94.11 ± 0.09	98.75 ± 0.24	99.1 ± 0.08
10	Viscosity at 4° C cP	4148 ± 3	8013.5 ± 2.5	6935 ± 1	6069.5 ± 0.5
11	Viscosity at 37° C cP	31504 ± 66.5	60041.25 ± 5.75	57551.25 ± 20.2	37015.25 ± 4.7

Table 3: Results of Formulation of Poloxamer-Chitosan-PVA Ophthalmic Gel

Sr No.	Formulation Code	CPXF5	CPXF6	CPXF7
	Evaluation			
1	Clarity Solution	Clear	Clear	Not Clear
2	Clarity Gel	Clear	Clear	---
3	Appearance Solution	Transparent	Transparent	Whitish
4	Appearance Gel	Transparent	Transparent	---
5	pH	7.1	7.3	---
6	Gelation Temp. (°c)	31.25±0.25	32.40±1.2	---
7	Gelation After Dilution (°c)	31.70±0.10	35.30±0.20	---
8	Gelation Capacity	+++	++	---
9	Drug Content (%)	96.98±0.12	95.80±0.52	---
10	Viscosity at 4°c cP	6814.5 ±0.5	6476±68	---
11	Viscosity at 37°c cP	56944.75±44.75	44910.25±29.75	---

In-Vitro Release Study (Cumulative % Released After 8 Hour (t_{8h}))

The cumulative percentage of ciprofloxacin released from the formulation as a function of time is shown in Fig.3. Ciprofloxacin release from optimized formulation was highly depends upon the concentration of polymers used. Drug release of poloxamer (CPXF1) was studied over combination of poloxamer-Chitosan (CPXF2). A study shows that active substance release from the formulation with fixed poloxamer concentration increases with the addition of Chitosan (0.2 %w/w). For the formulation containing poloxamer only (CPXF1) with shows 41.681% after 8 hour (t_{8h}), while poloxamer-Chitosan (CPXF2) shows increase in cumulative release up to 49.91% but not enough. An attempt was made in order to increase further release. Addition of PVP 2%w/w (CPXF3) shows significant increment in drug release (59.173%). Though CPXF3 shows considerable increment in percentage drug release was unable

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to give gelation temperature in the desired temperature range (35–37°c) so it was discarded. It was also observed that any increase in concentration of PVP from 0.20w/w or above failed in optimization of formulation due to failure in phase transition temperature at temperature 35-37°c. when PVP was replaced by HPC (CPXF4) shows declination in percentage drug release (51.31%). In next formulation, HPC was replaced by PVA (CPXF5). 0.2 %w/w PVA increases drug release up to 63.9%. This increase in percentage drug release with increase in PVA concentration shows flux enhancing action in gel formulation. In next formulation an attempt was made to increase the percentage drug release by keeping other parameters stable. PVA concentration was change from 0.2 to 0.4 %w/w. It was observed that increased PVA (CPXF6) concentration to 0.3% w/w results in further increase in cumulative drug release up to 71.06%. Further increase in PVA

(CPXF7) concentration does not show any phase transition.

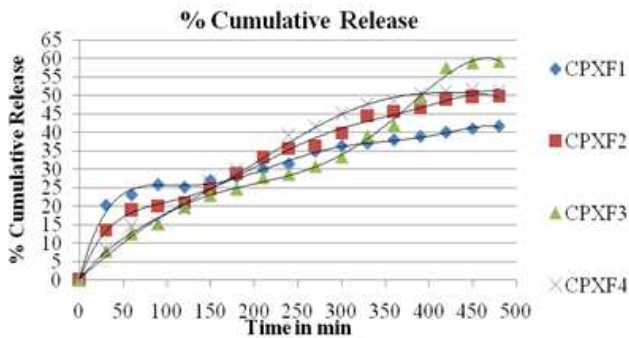


Fig.3: Cumulative % drug released for Poloxamer-Chitosan formulations

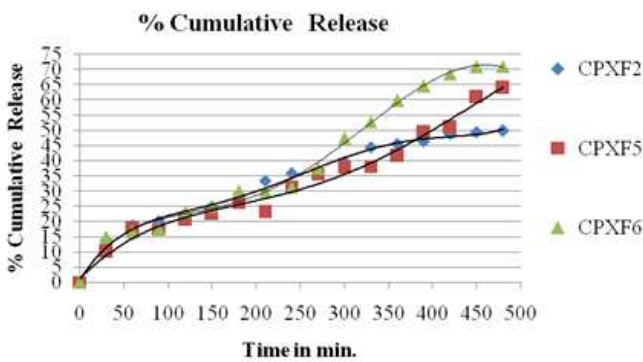


Fig.4: Cumulative % drug released for Poloxamer-Chitosan-PVA formulations

Rheological Studies

For thermosetting gels, the viscosity at various conditions is an important rheological parameter involved in its utilization and its in-vivo performance. For example, if viscosity is too high it will lead to difficult instillation; on the contrary, if viscosity is too low it will give rise to increased drainage.

Stability Studies

Stability studies - As per ICH Guidelines. Formulation were placed in ambient colored vials and sealed with aluminium foil for a short term accelerated stability study at 40±2 °C and 75±5% RH as per International Conference on Harmonization states Guidelines Samples were analyzed after every 15 days for period of 45 days for clarity, pH, gelling capacity, drug content and rheological evaluation. Data shown in fig 7, 8, 9 and 10.

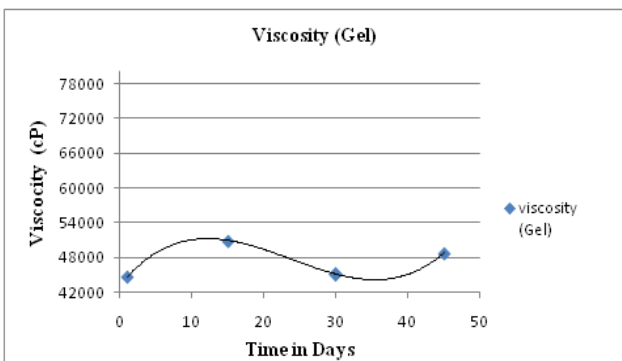


Fig 8: viscosity of gel

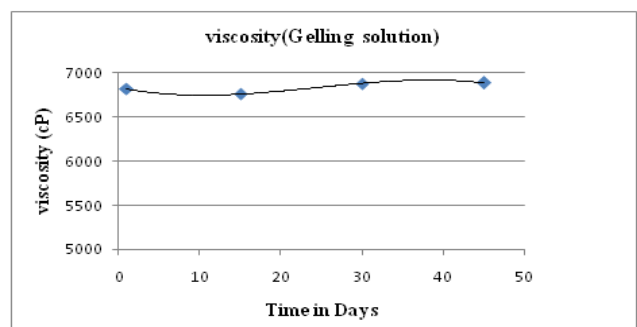


Fig 7: viscosity of gelling solution

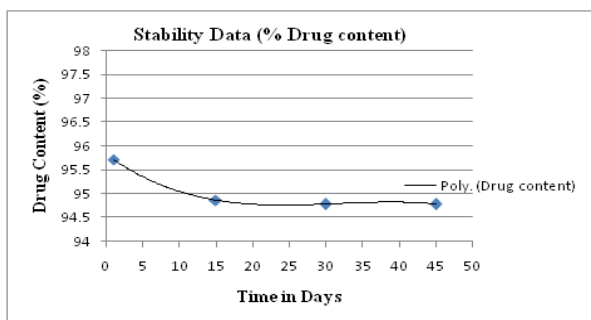


Fig 9: % Drug Content

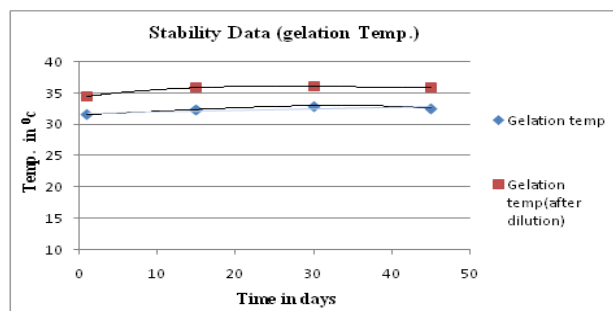


Fig 10: Gelation temperature

CONCLUSION & FUTURE PROSPECT

The novel environmentally responsive (thermoreversible) ophthalmic drug delivery system was successfully formulated by using natural poly-cationic copolymer with Poloxamer F 127. The formulated *in situ* gelling systems were characterized for appearance, clarity, pH, gelling capacity, phase transition temperature, rheological characteristics, *in vitro* release in simulated tear fluid. The formulation was liquid at the cold temp and underwent rapid gelation upon raising the temperature to 32°C. Ciprofloxacin HCl *in situ* gel (CPXF6) presents good stability. No macroscopical physical changes were observed during storage. Viscosity, pH, drug content, gelation temperature values of the formulation were carried out after every 15 days for the period of 45 days, shows no significant difference (data shown in fig. 7,8,9 and 10)

Thus, ophthalmic systems that deliver only the pure drug might allow for their use with greater safety. Finally, systems that provide continuous, prolong drug release to the eye may in time find important uses in the treatment of ophthalmic diseases. The provision of continuous ocular drug delivery to the eyes fix the problem of frequent instillation and drug loss due to several factors like naso-lacrimal drainage etc, Thus. *In situ* gel drug delivery system offers some hope for improving the epidemiological picture of severely debilitating eye diseases.

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