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Research Article

DESIGN OF POLYOXYETHYLENE-POLYOXYPROPYLENE BLOCK CO- POLYMER BASED IN SITU GELLING SYSTEM FOR LOCALIZED OCULAR DRUG DELIVERY

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ABSTRACT

Ocular drug delivery is limited both by patient acceptability and by the limited time that the dosage form is retained within the precorneal region. The timely gelation and retention of in situ-gelling ophthalmic formulations would be fundamental to improve the efficacy of drugs. The aim of this investigation was to develop ocular drug delivery system with simplicity in installation for patient, prolonged contact time with corneal epithelium with appropriate rheological properties. Formulation was evaluated on different parameters like pH, Drug content uniformity, gelation capacity, Measurement of phase change temperature, Isotonicity, Rheology, in-vitro drug release and stability studies.

Keywords: Insitu, Gelation, Poloxamer, In-Vitro Release Study.

INTRODUCTION

Topical delivery into cul-de-sac is, by far the most common route of ocular drug delivery. Whenever an ophthalmic drug is applied to the anterior segment of the eye, only small amount (5%) actually penetrates the cornea and reaches the interior tissue of the eyes [1].

Factors that affects drug bioavailability includes rapid solution drainage by Gravity, Induced lachrymation, Blinking reflex, Normal tear turnover. 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 [2]. Most successful have been enhancements of topical delivery by improvements to standard drop forms through use of gelling agents. Poloxamer is non-ionic surface active agent, and block copolymers consisting of polyoxyethylene-polyoxypropylene-polyoxyethylene units. Their relatively low toxicity and capacity to form clear gels make them particularly suitable for pharmaceutical applications, such as dermatological or ophthalmic formulations as well as in the area of controlled drug delivery systems. Gel formulations have found to be successful in achieving much better drug

product effectiveness, reliability and safety. In this regard, poloxamer is very useful with majority of hydro gels, which undergo reversible volume and/or sol-gel phase transitions in response to physiological (temperature) stimuli [3]. Ciprofloxacin HCl (1-Cyclopropyl-6-fluro-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 (Topioisomerase II) which mediate the formation of supercoils of DNA.

EXPERIMENTAL

Material

Poloxamer 407 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.

Methods

Formulation of Poloxamer-Chitosan Ophthalmic Gel

The formulations were prepared on a weight/weight basis using the cold method. In case of poloxamer only, certain volume of bidistilled water was cooled down to 4°C. Appropriate amounts of P407 were then slowly added to the cold water with continuous stirring. The dispersions were stored in a refrigerator at 4°C over night results in clear solution [68]. While In case of poloxamer and Chitosan, 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.

Formulation of Poloxamer-Chitosan-PVA Ophthalmic Gel

Appropriate amount of PVA was dissolved in bidistilled water, sonicated if necessary 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 121ºC and 15 psi for 20 min and then stored in the refrigerator prior to further evaluation. Formulation details were shown in Table 1.

Characterization of Ciprofloxacin Gel 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 (Motic DMWB B3 SERIES) at 40X magnification and compared with standard marketed ophthalmic formulation containing ciprofloxacin. The shape of blood cell was compared with standard marketed ophthalmic formulation containing Ciprofloxacin HCl.

Uniformity of Drug Content[7]

Drug content of ciprofloxacin formulations was determined by dissolving an accurately weighed quantity of formulation (100mg) 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 refrigerated tested ciprofloxacin 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 (or for 2 min when temperature was increased with an increment 0.1°C). The gelation is considered to be occurred when the meniscus of the formula would no longer move upon tilting through angle 90°. The maximum accepted gelation temperature tested was 34°C, which represents the corneal surface temperature. Each sample was measured at least in triplicate.

Effect of Dilution on Phase Transition Temperature[4]

The conventional commercial eye drop bottle delivers an average drop volume of about 40 ul, whereas the available tear fluid is 7 ul. 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 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. 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. 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.

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 4^0c and elevated temperature (37^0c). The developed poloxamer/Chitosan/PVA—ciprofloxacin formulations.

Sterility Testing[11]

IP method (1996) was followed for the sterility testing of eye drops. Sterility testing was carried out by incubating formulations for not less than 14 days at 30 to 35 °C in the fluid thioglycolate medium to find the growth of bacteria and at 20 to 25 °C in the soyabean-casein digest medium to find the growth of fungi in the formulations.

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) (soaked overnight 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 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. The amount of ciprofloxacin released at each time was calculated from a calibration curve.

Antimicrobial Efficiency of Controlled Release Ciprofloxacin Gel[7]

The antimicrobial efficiency and prolonged effect of selected controlled release ciprofloxacin gel were determined on staphylococcus strains. 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.

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 and table 3.The appearance of all the gelling solutions and gel formed were transparent and clear with few exceptions like CPXF3and CPXF6. Out of which CPXF6 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 98.75%. pH of all the formulation were in at 7.3 ±0.3.

Isotonicity Evaluation

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 Figure.1 and Figure 2).

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 Figure.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 HPC 2%w/w (CPXF3) shows significant increment in drug release (58.912%) but unable to show phase transition temperature in desired range. when HPC was replaced by PVA, further increment in percentage drug release was observed. In next formulation an attempt was made to increase the percentage drug release by keeping other parameters stable. PVA concentration was change from 0.3 to 0.4 %w/w. It was observed that increase 0.2% w/w PVA (CPXF4) concentration to 0.3 %w/w (CPXF5) shows further increment up to 68.71%. This increase in percentage drug release with increase in PVA concentration shows flux enhancing action in gel formulation. Further increase in PVA (CPXF7) concentration does not show any phase transition.

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 2 and 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 (CPXF1) 20 % w/w concentration shows phase transition temperature of 28.75±0.25°c.

Pre-formulation studies show 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 PVA above 0.3% w/w to poloxamer formulation fails for gelation (CPXF6). All the formulations show gelation temp in an acceptable range (28.7 °c to 32.4 °c) that is the temp of cul de sac. It was observed that after dilution with simulated tear fluid (40:7) phase transition temperature increases. Gelation temperature after dilution for all the formulation was found in the range of 31.3 °c to 36.3 °c. Details for each formulation for effect of dilution have shown in Figure. 4.

Antimicrobial Efficiency of Controlled Ciprofloxacin HCl Gel Formulations

The result of the antimicrobial efficacy tests shown in table 4. The ZOI values for the prepared formulations were higher than the ZOI values of the standard preparation (Figure 5). 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.

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. The gels formed at physiological conditions, and the viscosity of the formulations decreased as the shear rate increased, which showed the character of pseudoplastic fluid. Because the ocular shear rate is very large ranging from 0.03/second (during interblinking periods) to 28,500/second during blinking, viscoelastic fluid with a viscosity that is high under low shear rate conditions and low under high shear rate conditions is preferred. All the formulations show the viscosity in the range of 31504 to 60041 cP for physiological condition and 4148 to 8013 cP to for non-physiological condition. Data showed at table 2 and 3.

Stability Studies

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 ICH Guidelines. Samples were analyzed after every 15 days for period of 45 days for clarity, pH, gelling capacity, drug content and rheological evaluation. Optimized formulation CPXF6 presents good stability. No macroscopial physical changes were observed during storage. Viscosity, pH, drug content, gelation temperature values of the formulation does not shows any significant difference (data shown in Figure 6, 7, 8 and 9).

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

Formula Variation (%w/w)	CPXF1	CPXF2	CPXF3	CPXF4	CPXF5	CPXF6
Ciprofloxacin HCl	0.30	0.30	0.30	0.30	0.30	0.30
Poloxamer	20	20	20	20	20	20
Chitosan	---	0.20	0.20	0.20	0.20	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
Triethanolamine	qs	Qs	qs	qs	qs	qs
Benzalkonium Chloride	0.001	0.001	0.001	0.001	0.001	0.001
Purified water	q.s. 100	q.s. 100	q.s. 100	q.s. 100	q.s. 100	q.s. 100

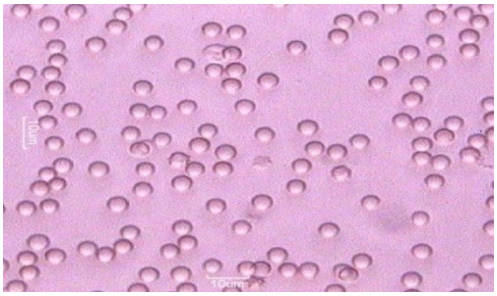

Fig. 1: Marketed Formulation

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

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

S. No.	Formulation Code / Evaluation	CPXF1	CPXF2	CPXF3
1	Clarity Solution	Clear	Clear	Not Clear
2	Clarity Gel	Clear	Clear	Whitish
3	Appearance Solution	Transparent	Transparent	Cloudy
4	Appearance Gel	Transparent	Transparent	Cloudy
5	pH	6.8	7.1	7.4
6	Gelation Temp.	28.75±0.25	32.30±0.20	29.20 ±0.10
7	Gelation After Dilution	31.35±0.15	36.35±0.25	32.55 ±0.15
8	Gelation Capacity	++	++	+++
9	Drug Content (%)	90.80±0.77	94.11±0.09	98.75+0.24
10	Viscosity at 4 °c cP	4148±3	8013.5±2.5	6935±1
11	Viscosity at 37 °c cP	31504±66.5	60041.25±5.75	57551.25±20.2

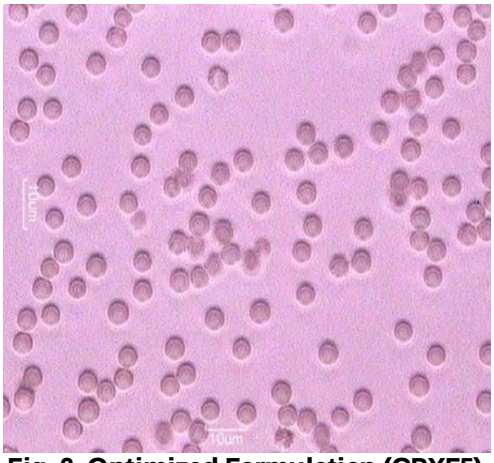

Fig. 2: Optimized Formulation (CPXF5)

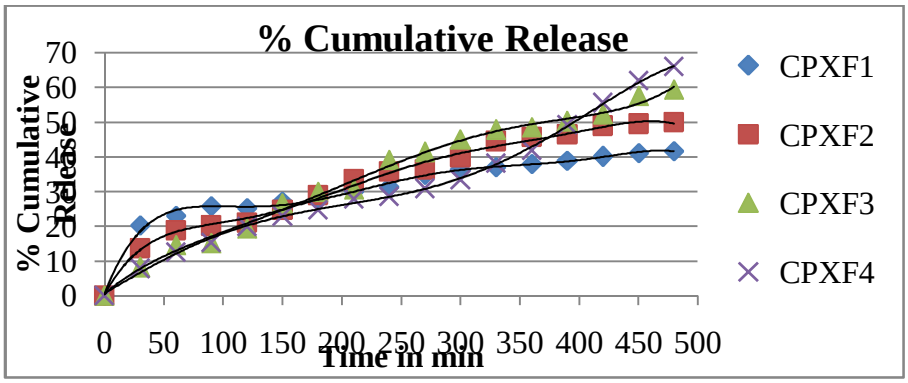

Fig. 3: Cumulative % Drug Released for Poloxamer-Chitosan Formulations

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

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

S. No.	Formulation Code / Evaluation	CPXF4	CPXF5	CPXF6
1	Clarity Solution	Non Clear	Clear	Not Clear
2	Clarity Gel	Non Clear	Clear	---
3	Appearance Solution	whitish	Transparent	Whitish
4	Appearance Gel	whitish	Transparent	---
5	pH	---	7.3	---
6	Gelation Temp.	---	32.40 ±1.2	---
7	Gelation After Dilution	---	35.50 ±0.20	---
8	Gelation Capacity	---	++	---
9	Drug Content (%)	---	95.80±0.52	---
10	Viscosity at 4 °c cP	---	6476±68	---
11	Viscosity at 37 °c cP	---	44910.25±29.75	---

Table 4: Zone of Inhibition (ZOI) for S. Aureus

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

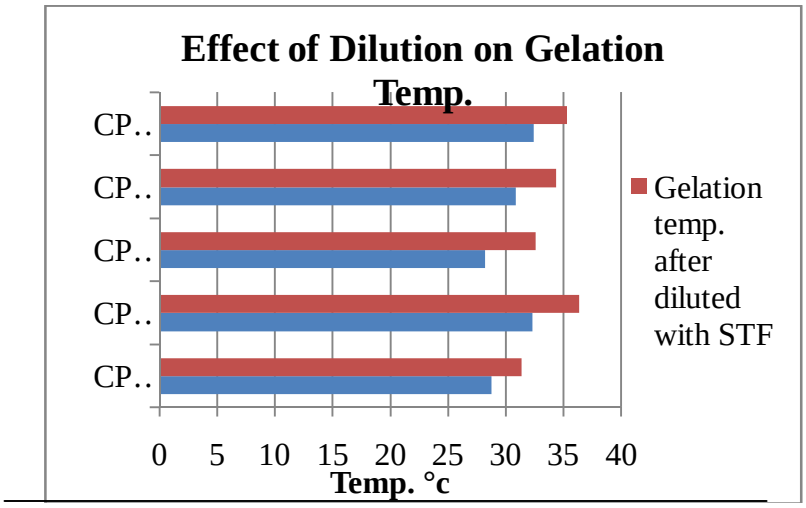

Fig. 4: Effect of Dilution on Gelation Temperature for Chitosan-poloxamer Formulation

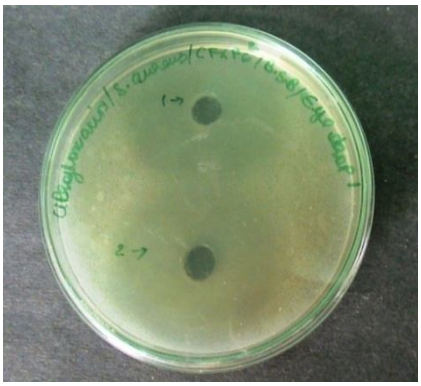

Fig. 5: Antimicrobial activity against s. aureus

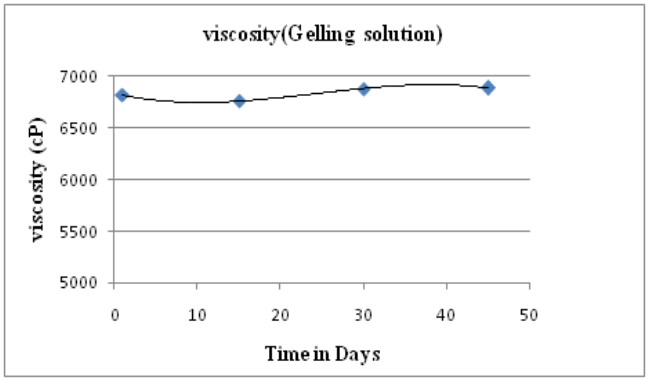

Fig. 6: Viscosity of Gelling Solution

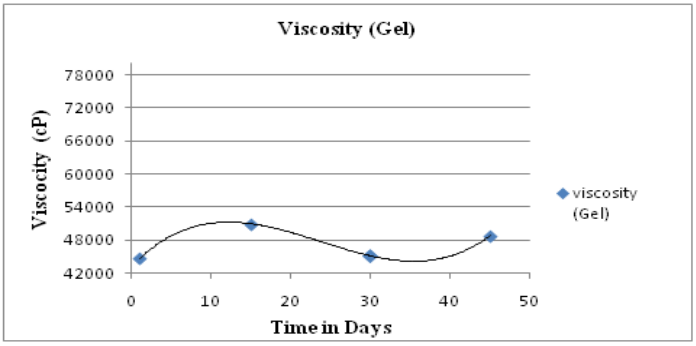

Fig. 7: Viscosity of Gel

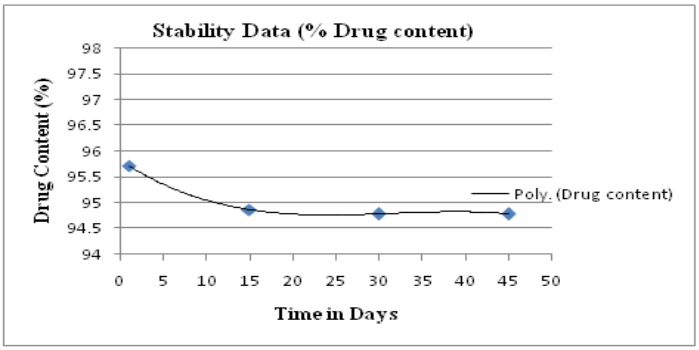

Fig. 8: % Drug Content

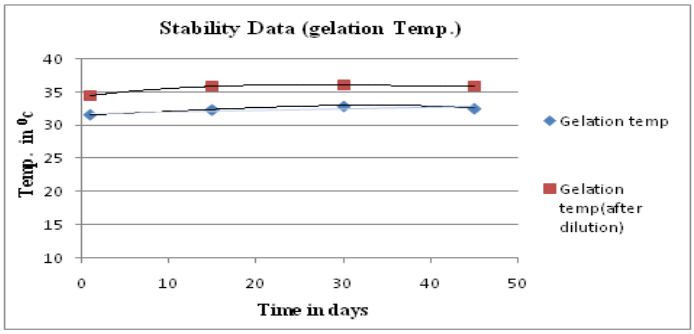

Fig. 9: Gelation Temperature

CONCLUSION

The novel thermoreversible ophthalmic *in situ* gelling drug delivery system was successfully formulated by using Poloxamer F 127, Chitosan and PVA The formulated *in situ* gelling systems were characterized for appearance, clarity, pH, gelling capacity, phase transition temperature, Gelation temperature after dilution with STM, rheological character, in vitro release in simulated tear fluid. The formulation was liquid at the cold temp (8.0) and underwent rapid gelation upon raising the temperature to 32°c. Ciprofloxacin HCl in situ gel (CPXF6) presents good stability. No macroscopial 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 Figure. 6, 7,8,and 9)

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. Insitu gel drug delivery system offers some hope for improving the epidemiological picture of severely debilitating eye diseases.

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