

TEMPERATURE SENSITIVE OPHTHALMIC HYDROGELS OF LEVOFLOXACIN HEMIHYDRATE WITH ENHANCED SOLUBILITY AND PROLONGED RETENTION TIME

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Received: 11 July 2013, Revised and Accepted: 02 Aug 2013

ABSTRACT

Objective- The prolonged retention of drug formulation in the eye is of utmost importance for topical ophthalmic drug delivery. The objective of the present investigation is to develop mucoadhesive Temperature Sensitive Ophthalmic Gels of Levofloxacin hemihydrate, a Fluoroquinolone antibiotic drug with enhanced solubility and prolonged retention time in order to improve the bioavailability of drug.

Methods- The gelation upon contact with conjunctival membrane was conferred via the use of the Thermogelling Poloxamer 407 whereas mucoadhesion and drug release enhancement were modulated via the use of mucoadhesive and polyethylene glycol (PEG) polymers respectively. β -Cyclodextrin in 0.2% concentration was used to increase the solubility of Levofloxacin.

Results- The results revealed that the different Mucoadhesives augmented the retention time but extensively increased the viscosity of formulations due to which the formulations were too viscous to be instilled and may cause blurring of vision after instillation. The inclusion of PEG counteracted the effect of the mucoadhesive polymers whereby it decreased the viscosity to desired level and increased the *in vitro* drug release. The formulations with favorable sol-gel transition temperatures (35–37°C), Viscosity and high *in vitro* drug release were also therapeutically efficacious, sterile, stable, non irritant and rheologically stable upon storage. Formulations provided sustained release of the drug over a period of 6 h.

Conclusion- The study points to the potential of Temperature Sensitive Ophthalmic Gels in terms of ease of administration, accuracy of dosing, prolonged retention and improved drug bioavailability.

Keywords: Ophthalmic; Temperature sensitive; Hydrogel; Levofloxacin; Instillation; Viscosity.

INTRODUCTION

Eye is one of the challenging organs for drug delivery because its unique anatomy restricts drug absorption into the deep tissues. Although various ophthalmic drug delivery systems such as inserts, ointments and suspensions have been developed to overcome this problem, they have not been accepted by patients due to several drawbacks i.e., difficult administration of inserts, blurred vision from use of ointments and dosage heterogeneity of suspensions [1]. Consequently, more than 90% of marketed ophthalmic formulations are still in a form of an eye drop of water soluble drugs [2]. The main reason for using the eye drop is simple instillation into the eye with accuracy doses [3, 4]. However, this conventional system could not be considered as an optimum formulation for treatment of eye disorders due to the rapid precorneal elimination by protective mechanisms of the eye such as blinking reflex, lacrimal fluid dilution and nasolacrimal duct drainage [1]. In order to avoid the rapid dilution, formulations with an increased viscosity have been evaluated. Among them, the *Stimuli sensitive* gel-forming formulations, which undergo phase transition from liquid to semisolid gel upon exposure to physiological stimuli such as Temperature, pH and ionic interactions, seem to be a promising tool. In particular, a Temperature sensitive gel, is an ophthalmic product vehicle responding to a shift in temperature, possesses liquid characteristic at low temperature and becomes gel when comes into contact with a certain temperature defined as a sol-gel transition temperature. These formulations should be capable of withstanding shear forces in the cul-de-sac and of sustaining drug release at physiological conditions [5].

Thermosensitive amphiphilic block copolymers, namely poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (PEO-PPO-PEO, Poloxamer), have been extensively investigated as Thermosensitive gelling agents [6–11]. They show excellent thermosensitive gelling properties, low toxicity and irritation, excellent water solubility, good drug release characteristics and compatibility with other chemicals [12–17]. The most accepted mechanism to explain the thermogelification of Poloxamer is that, as the temperature is increased, polymer desolvation and subsequently micellization occur forming more closely packed viscous gel. The

driving forces for the micellization and the accompanied expulsion of the hydrating water from the core of the micelles are the conformational changes in the orientation of the methyl groups in the (PPO) side chains of the polymer [18–23]. Though Poloxamer are widely employed, they suffer from a major drawback of having weak mechanical strength, which leads to rapid erosion [24]. One interesting approach, however, focuses on blends of Poloxamer with several mucoadhesion polymers like Carbopol and Poly vinyl alcohol etc. [4]. These polymers increase the formulation's mechanical strength, but also increase surface interaction with the ocular tissue and, consequently, contact time. Various studies have revealed that the different Mucoadhesives increase the viscosity of formulations extensively due to which the formulations become too viscous to be instilled and may cause blurring of vision after instillation. The inclusion of other polymers like Polyethylene Glycol counteracts the effect of the mucoadhesive polymers whereby it decreases the gel viscosity to a desired level and increase the *in vitro* drug release.

The aim of the present study is to develop Temperature sensitive Hydrogels of an Antibacterial drug **Levofloxacin Hemihydrate** using Poloxamer 407 as thermosensitive gelling agent and other polymers such as Carbopol 940P, PVA and PEGs in order to increase the precorneal residence time & bioavailability of drug.

Levofloxacin (LVFX) is a third generation fluoroquinolone antibacterial agent with a broad spectrum of activity against Gram-positive and Gram-negative aerobic bacteria and atypical bacteria, and limited activity against most anaerobic bacteria. It exerts its antibacterial effects by inhibiting bacterial DNA gyrase and topoisomerase IV. It is indicated for the treatment of acute conjunctivitis.

LVFX is well absorbed with bioavailability of approximately 99%. Its volume of distribution is about 1.1 L/kg and protein binding is 24–38%. Levofloxacin is stereochemically stable in plasma and urine and does not invert metabolically to its enantiomer, D-Ofloxacin. It is excreted through the kidneys with 64–102% of the dose as unchanged drug. The half-life of LVFX is between 6–9 hours [25].

But the use of Levofloxacin in ophthalmic formulations is limited due to its pH-dependent solubility. Over the pH range of 2–5, the

solubility is essentially constant, above pH 5.5, the solubility increases to a maximum value at about pH 6.2, whereas above pH 6.2, the solubility decreases and reaches its minimum value. Since an ophthalmic formulation must have a pH in the range of 6.2-7.4, hence most of the Levofloxacin Ophthalmic formulations suffer the drawback of either poor bioavailability or frequent administration. But if Levofloxacin is formulated along with some Solubility enhancer like β -Cyclodextrin, Its solubility is found to increase to a remarkable extent at the pH above 6.2 also.

Hence Temperature sensitive Hydrogels of Levofloxacin hemihydrate was formulated along with 0.2% concentration of β -Cyclodextrin in view of increasing the bioavailability of the formulations and reducing the frequency of administration.

MATERIALS AND METHODS

Materials

Levofloxacin hemihydrate was obtained as a gift sample from Galpha Laboratories Ltd., Baddi (Himachal Pradesh). Poloxamer 407 was obtained from Zeil Pharmaceuticals, Mumbai while rests of the Polymers were obtained by SD Fine Chemical Ltd., Mumbai. All chemicals used were of analytical grade.

Table 1: Table shows formulation design for Preparation of Temperature Sensitive Hydrogels

Contents	Quantity (% w/w)				
	TF1	TF2	TF3	TF4	TF5
Drug	0.512	0.512	0.512	0.512	0.512
Poloxamer 407	18	20	18	18	18
Poly ethylene glycol 6000	1	1	-----	-----	-----
Poly ethylene glycol 400	-----	-----	8	8	6
Poly vinyl alcohol	0.5	0.5	0.5	-----	-----
Carbopol 940	-----	-----	-----	0.3	0.3
β -Cyclo Dextrin	0.2	0.2	0.2	0.2	0.2
Benzalkonium chloride	0.1	0.1	0.1	0.1	0.1
Sodium chloride	0.9	0.9	0.9	0.9	0.9
Phosphate buffer pH 7.4	QS100 gm	QS100 gm	QS 100 gm	QS100 gm	QS 100 gm

Method of Preparation

The cold method described by Schmolka (1972) [26] was adopted. Briefly, the drug, Poloxamer 407, PEG and/or mucoadhesive polymers were stirred in the calculated amount of 7.4 pH phosphate buffer at room temperature. The dispersions were cooled down to 4°C; the Poloxamer dispersion was added slowly with continuous stirring (thermostatically controlled magnetic stirrer, Navyug India) to drug and polymers dispersion. The dispersion was then stored in a refrigerator until clear solutions was obtained. The developed formulations were filled in 25-ml capacity amber glass vials, closed with gray butyl rubber closures and sealed with alu-minium caps. The formulations, in their final pack, were then sterilized by autoclaving for 30 minutes at 121°C temperature and 15 psi pressure.

Evaluation of Formulations

Appearance, Clarity, pH and Percent drug content

The appearance and clarity were determined by visual observation against a black and white background. The pH of the formulations was determined by using Elico India Ltd. digital pH meter. The Percent drug content of Temperature Sensitive hydrogel was determined by taking sample (1ml) of in-situ gel in a 100ml volumetric flask. Aliquot of 1 ml was withdrawn and diluted with simulated tear fluid of pH 7.4 to 10 ml. The absorbance was measured at λ max 287 nm using UV-spectrophotometer (UV-1700 Pharma Spec, Shimadzu, Kyoto, Japan) to calculate the percentage of drug content.

Gelling capacity

All Temperature Sensitive gelling formulations were evaluated for gelling capacity to identify the compositions suitable for use. The

Identification

For the confirmation of purity of drug, the given sample of Levofloxacin hemihydrate was subjected to various identification tests such as, melting point, solubility, λ_{max} determination, FTIR Spectroscopy and Powder X-Ray Diffractometry etc.

Compatibility Studies

Fourier Transform Infrared (FTIR) Spectroscopy studies were used for the evaluation of physicochemical compatibility and interactions of the Levofloxacin hemihydrate with polymers. The FT-IR spectra of Physical mixtures of Levofloxacin hemihydrate with polymers were compared with the standard FT-IR spectrum of the pure drug.

Preparation of Temperature Sensitive Hydrogel formulations

Various Combinations of Poloxamer 407, Polyethylene glycol 6000, Polyethylene glycol 400, Polyvinyl Alcohol and Carbopol 940 in different concentrations were prepared and evaluated by visually assessing the gelling capacity and viscosity in order to identify the composition suitable for use as in Temperature Sensitive systems. Formulations were prepared according to the Formulation design given in **Table-1**.

gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of Simulated tear fluid freshly prepared and equilibrated at 37°C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve. The composition of simulated tear fluid used was sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride dihydrate 0.008 g and purified water Q.S. 100.0 g.

Viscosity Studies of Liquid Formulations [27]

All Temperature Sensitive gelling formulations in liquid form were evaluated for Viscosity. Viscosity of liquid formulations was measured using a Brookfield Synchroelectric viscometer (DV- II +Pro) in the small volume adapter. The viscosity of liquid formulations measured at 10 rpm was used for purposes of comparative evaluation of prepared Temperature Sensitive hydrogel formulations.

In vitro Release Studies

The in vitro release of Levofloxacin hemihydrate from all selected Temperature Sensitive formulations was studied through cellophane membrane using a USP-I dissolution testing apparatus. The dissolution medium used was Simulated Tear Fluid freshly prepared (pH 7.4). A 2-ml volume of the gelled formulation was accurately kept in Cellophane membrane, previously soaked overnight in the dissolution medium to form a cellophane pouch. Cellophane membrane pouch having drug was put in the cylindrical basket. The cylindrical basket was attached to the metallic driveshaft and suspended in 900 ml of dissolution medium maintained at 37± 1°C. The dissolution medium was stirred at 50 rpm. Aliquots, each of 5-ml volume, were withdrawn at regular intervals and replaced by an equal volume of the dissolution medium. The aliquots were diluted with the dissolution medium and analyzed by UV-Vis spectrophotometer at 287 nm.

Drug Release Kinetics studies

Drug release kinetics was studied by curve fitting method to different kinetic models of zero order, first order, Higuchi and Hixson Crowell models To find out the mechanism of drug release, 60 % drug of release data was first fitted in the Korsmeyer-Peppas model and the value of 'n' the diffusion exponent was determined. According to this model if 'n' is b/w 0.45 to 0.5 the Fickian mechanism, 0.5 to 0.8 the Non-Fickian and if 0.8 to 1.0 Case-II transport i.e. a zero-order mechanism is governing the drug release mechanism from the gels.

Viscosity Studies of Gelled Formulations

Viscosity of instilled formulation is an important factor in determining residence time of drug in the eye. It was noted from various literature that the formulations before gelling should have a viscosity of 5 to 1000 mpa and after gelling in the eye will have a viscosity from about 50-50,000 mpa. The selected Temperature sensitive formulations were allowed to gel in the simulated tear fluid and then the viscosity determination were carried out by using Brooke field viscometer (DV-II +Pro) at angular velocity of 10 rpm.

Sterility Testing

IP method (1996) was followed for the sterility testing of Temperature sensitive eye drops. Sterility testing was carried out by incubating formulations for not less than 15 days at 30 to 35° C and 75% relative humidity in the Liquid Nutrient broth media.

Pyrogen Testing

An ophthalmic formulation must be Pyrogen free. After sterilization the end products or metabolic products of bacteria might be left in formulation, which may act as Pyrogen. Selected Temperature Sensitive formulations were subjected to Qualitative Fever Response Test in rabbits. Pyrogen testing was performed according to IP. Three rabbits weighing 2.25 kg were selected for the purpose. Formulation in a dose of 10ml/kg of body weight was injected in the ear vein of rabbit and injection was completed within 10 seconds. Rectal temperature after giving the formulation was recorded at 1, 2 and 3 hr. and Rise in temperature was determined.

Ocular Irritation Studies

Ocular irritation studies were performed on two male albino rabbits each weighing 2.25 kg. The selected sterile Temperature Sensitive formulations were instilled twice a day for a period of 21 days and

the rabbits were observed periodically for redness, swelling and watering of the eye.

Accelerated Stability Studies

All selected Temperature Sensitive formulations were subjected to stability studies conditions at 4°C, room temperature (25°C) with ambient humidity, 37°C with 80% relative humidity and 60°C for a period of one month. The samples were withdrawn after 7, 15 and 30 days and were evaluated for Appearance, clarity, pH and Drug content.

RESULTS

Identification

Melting point, solubility, λ_{max} determination, FTIR Spectroscopy and Powder X-Ray Diffractometry confirmed the purity of Levofloxacin hemihydrate. Fig.1 shows the PXRD pattern of Levofloxacin hemihydrate.

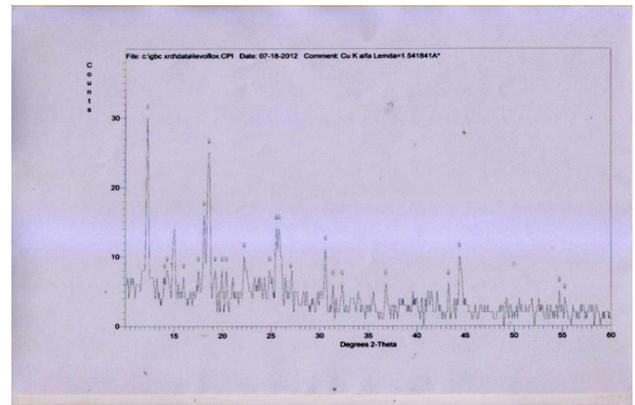


Fig. 1: It shows Powder X-ray Diffraction Pattern of Levofloxacin hemihydrate

Compatibility studies

The individual IR spectra of the pure drug and Physical mixtures of Levofloxacin hemihydrate with polymers (Fig.2) indicated no interaction between Levofloxacin and polymers when compared with infrared spectrum of pure drug, as all functional group frequencies were present.

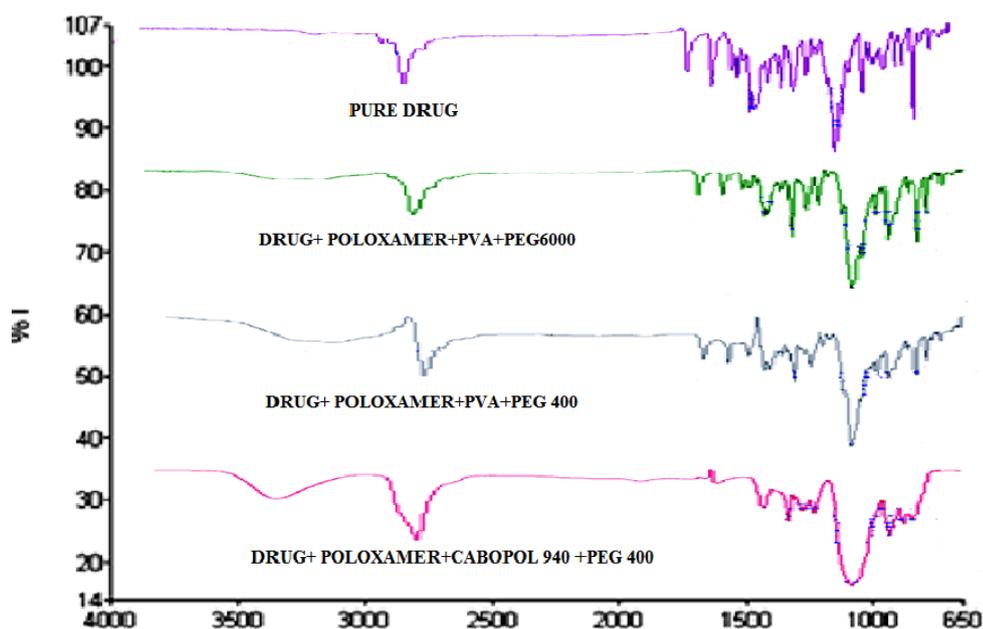


Fig. 2: It shows drug polymer compatibility study

Preparation of Temperature Sensitive Ophthalmic Hydrogels

5 Formulations containing Drug and Polymers in different concentrations were prepared. The use of Poloxamer 407 in Temperature Sensitive Hydrogels is substantiated by the property of its aqueous solutions to transform into stiff gels when the temperature is changed from room temperature to 37°C. Carbopol 940 and PVA increased the viscosity of formulation while PEGs contributed in maintaining the consistency of formulations. The phosphate buffer pH 7.4 was used as a vehicle in the Temperature Sensitive gelling system. (Fig.3)



Fig. 3: It shows Temperature sensitive hydrogel formulations in liquid and gelled form

Evaluation

Appearance, Clarity, pH and Percent drug content

The appearances of all formulations were light yellow in colour and were clear. Terminal sterilization by autoclaving had no effect on the formulations. The pH of all the formulations was found to be within

the range of 7.0 to 7.3, which is desirable for the ophthalmic formulations. The drug content of all the formulations was within the range of 95.05% to 97.90%, showed the uniform distribution of drug in the ophthalmic formulations (Table-2).

Gelling capacity

The main prerequisite of a Stimuli Sensitive gelling system is gelling capacity (speed and extent of gelation). The formulation should have an optimum gelling capacity, so that after instillation into the eye as a liquid (drops), it would undergo a rapid sol-to-gel transition and would preserve its integrity without dissolving or eroding for a prolonged period of time.

Table-2 shows the gelling capacity of all Temperature Sensitive formulations. All the formulations showed instantaneous gelation when contacted with simulated tear fluid (STF). Formulation TF1 and TF5 showed immediate gelation and remained for few hours, whereas the formulation TF2, TF3 and TF4 showed immediate gelation and remained for extended period.

Viscosity Studies of Liquid Formulations

An ophthalmic formulation must have an optimum viscosity that will allow easy instillation into the eye as a liquid (drops), which would undergo a rapid sol-to-gel transition. Table-2 shows the Viscosity of all liquid Temperature Sensitive formulations at 10 rpm. Results showed that the Formulations containing PEG 400 and Carbopol have more viscosity in comparison to Formulations containing PEG 400 and Poly vinyl Alcohol. However the Formulation containing PEG 6000 and Poly vinyl alcohol showed lesser viscosity than formulation containing PEG 400 and Poly vinyl alcohol. It indicated that PEG 6000 reduced the viscosity to a greater extent than PEG 400.

On the basis of gelling capacity and viscosity Formulations TF2, TF3 and TF4 showed optimum results within the desired range. Hence, these three Temperature Sensitive formulations were subjected for further evaluation parameters.

Table 2: Table shows Appearance, Clarity, pH, Drug Content, Gelling Capacity and Viscosity for liquid Formulations

Temperature Sensitive Hydrogels	Appearance	Clarity	pH	%Drug Content \pm S.D	Gelling Capacity	Viscosity at 10rpm (Pa-s)
TF1	Light Yellow	Clear	7.2	97.40 \pm 0.85	++	1.876
TF2	Light Yellow	Clear	7.3	95.05 \pm 0.21	+++	3.645
TF3	Light Yellow	Clear	7.0	97.30 \pm 0.85	+++	3.954
TF4	Light Yellow	Clear	7.2	97.90 \pm 0.42	+++	4.382
TF5	Light Yellow	Clear	7.3	95.15 \pm 0.92	++	2.205

TF2- Poloxamer 20% w/w, PEG (6000) 1% w/w, PVA 0.5%w/w; TF3- Poloxamer 18% w/w, PEG (400) 8% w/w, PVA 0.5%w/w; TF4- Poloxamer 18% w/w, PEG (400) 8% w/w, Carbopol 0.3%w/w.

+, Gels after a few minutes, dissolves rapidly; ++, Gelation immediate, remains for few hours; +++, Gelation immediate, remains for extended period.

Table 3: Table shows *In-vitro* Release study of Levofloxacin Hemihydrate

Time (Hrs)	Percent Drug Released (%)		
	TF2	TF3	TF4
0.25	10.03	13.95	13.95
0.5	15.11	21.78	21.78
1	22.41	28.97	28.97
1.5	28.87	36.69	36.69
2	35.00	41.25	41.25
2.5	40.08	48.34	48.34
3	46.75	53.52	53.52
3.5	52.36	59.45	59.45
4	59.02	64.63	64.63
4.5	65.05	70.13	70.13
5	71.19	77.96	77.96
5.5	79.87	82.72	82.72
6	87.91	88.22	88.22

TF2- Poloxamer 20% w/w, PEG (6000) 1% w/w, PVA 0.5%w/w; TF3- Poloxamer 18% w/w, PEG (400) 8% w/w, PVA 0.5%w/w; TF4- Poloxamer 18% w/w, PEG (400) 8% w/w, Carbopol 0.3%w/w

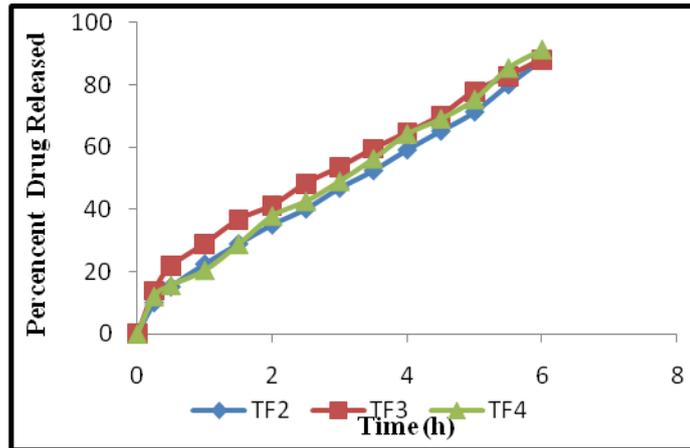


Fig. 4: It shows *in Vitro* Release Profile of Levofloxacin hemihydrate from selected Temperature Sensitive Hydrogel Formulations

In Vitro Release Studies

The *in-vitro* release profile of the formulations is shown in Table 3. The results indicated that the formulation TF4 showed better sustaining effect amongst all formulations. This may be due to the presence of higher concentration of PEG 400 along with Carbopol 940 in the formulation PF8. The *in vitro* drug release conditions may be very different from those likely to be encountered in the eye. However, the results clearly showed that the gels have the ability to retain drug for prolonged period of time (6 hour) and that

premature drug release will not occur. In vitro drug release profile for Temperature Sensitive Hydrogels is depicted in Fig. 4.

Drug Release Kinetics Studies

For all temperature sensitive formulations, the best fit model was Zero order while 'n' exponent value is b/w 0.5 to 0.8 indicating that formulations are released by Nonfickian diffusion mechanism or by anomalous diffusion in a controlled manner. The results are shown in Table 4.

Table 4: Table shows Model fitting for the Release Profile of Temperature Sensitive Formulations by Using 5 Different Models

Temperature Sensitive Formulations	Zero Order R	First Order R	Higuchi Matrix R	Korsmeyer - Peppas R	Hixson-Crowell n	Hixson-Crowell R	Best Fit Model	Transport Mechanism
TF2	0.998	0.952	0.982	0.998	0.630	0.978	Zero	Non Ficknian
TF3	0.997	0.973	0.992	0.998	0.530	0.989	Zero	Non Ficknian
TF4	0.999	0.948	0.982	0.985	0.597	0.977	Zero	Non Ficknian

TF2- Poloxamer 20% w/w, PEG (6000) 1% w/w, PVA 0.5%w/w; TF3- Poloxamer 18% w/w, PEG (400) 8% w/w, PVA 0.5%w/w; TF4- Poloxamer 18% w/w, PEG (400) 8% w/w, Carbopol 0.3%w/w

Viscosity Studies of Gelled Formulations

Fig. 5 shows the viscosity values obtained for selected Temperature Sensitive (TF2, TF3 and TF4) formulations respectively after gelling, using Brookfield Viscometer (DV- II +Pro) at the angular velocity of

10 rpm. All Viscosity values were found less than 50,000 mpa which is an optimum viscosity range for gelled ophthalmic formulations. Viscosity study showed that at room temperature, the formulations were in a liquid state and exhibited low viscosity. After instillation into eye they got transformed into gels with high viscosity.

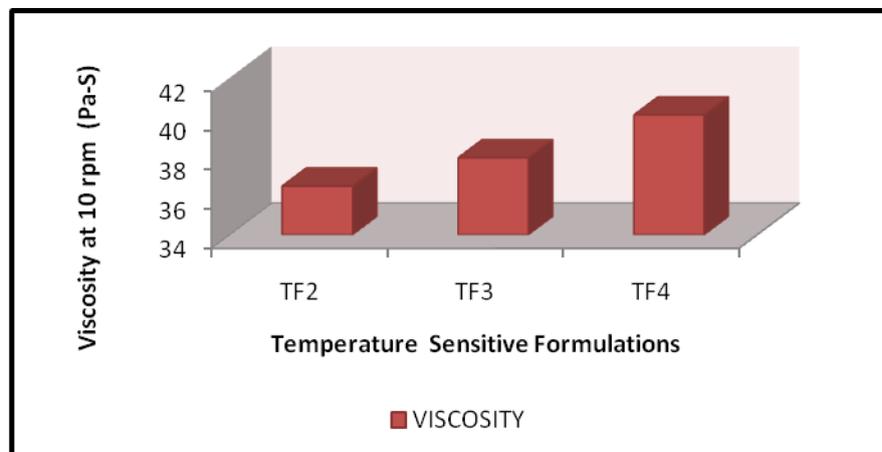


Fig. 5: It shows Viscosity Study of gelled Temperature Sensitive Formulations (At 10 rpm)

TF2- Poloxamer 20% w/w, PEG (6000) 1% w/w, PVA 0.5%w/w; TF3- Poloxamer 18% w/w, PEG (400) 8% w/w, PVA 0.5%w/w; TF4- Poloxamer 18% w/w, PEG (400) 8% w/w, Carbopol 0.3%w/w

Sterility Testing

There was no appearance of turbidity and hence no evidence of microbial growth when the formulations were incubated for 15 days at 37°C temperature and 75% relative humidity in Liquid Nutrient broth media. The preparations being examined therefore passed the test for sterility.

Pyrogen Testing

Pyrogen Testing showed that the average rise in temperature for individual rabbits was less than 0.6 for all Temperature sensitive formulations. Hence all formulations passed the Pyrogen test.

Ocular Irritation Studies

The results of the ocular irritation studies indicated that all Temperature sensitive hydrogel formulations were non-irritant. Excellent ocular tolerance was noticed for all the three formulations. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae were visible. No signs of redness, watering of the eye and swelling were observed throughout the study with both the formulations

Accelerated Stability Studies

From the results it has been observed that the formulations showed no or least changes in appearance, clarity, pH and Drug Content. Further it was observed that the gelling capacity of the formulations was least affected. Hence stability studies confirmed that Temperature Sensitive Hydrogel formulations of Levofloxacin hemihydrate remained stable at ambient temperature (25°C) and humidity.

DISCUSSION

The physiological temperature for the eye lies between 35 and 37°C. In the present study our target was to develop an Ophthalmic Temperature sensitive hydrogel of Levofloxacin Hemihydrate with a phase transition temperature between 32 and 37 °C. It would then behave as a liquid at the room temperature for ease of administration and accurate measurement of the dose but sets into a gel with increased residence time at the lower limit of the ophthalmic physiological temperature range. The loading of Levofloxacin hemihydrate in the different formulations was kept at 5% (w/w) such that 100µl gel (the optimum volume for ophthalmic instillation) would contain 5mg which is the adult dose. To increase the solubility of Levofloxacin hemihydrate β-cyclodextrin (0.2% w/w) was added. Poloxamer in a concentration of 18-25% w/w was used as Thermoresponsive gelling agent and to prolong the residence time Carbopol 940 or PVA were used as Mucoadhesives. However these polymers cause modification of the process of micellar association of Poloxamer gels thereby increase their viscosity. The viscosity increasing effect of mucoadhesive polymers could be explained by their ability to bind to polyethylene oxide (PEO) chains present in the Poloxamer molecules promoting dehydration and causing an increase in entanglement of adjacent molecules with more extensive intermolecular hydrogen bonding [28-30]. Thus inclusion of mucoadhesive polymers leads to excessively increased viscosity of formulations at room temperature as well as in gelled form, which makes the formulation difficult to instill as well as might cause blurring of vision after instillation. Therefore to maintain the optimum viscosity of Formulations, PEG 400 or PEG 6000 were added. The lowering of viscosity observed upon addition of PEGs could be attributed to the interference of PEG with the process of micellar association of Poloxamer chains.

Thus by varying the concentration of drug and polymers five formulations were prepared, which were then subjected to various evaluation parameters like Appearance, pH, drug content, *in vitro* gelation, *in vitro* viscosity of liquid formulation, *in vitro* drug release, *in-vitro* viscosity of gelled formulations, sterility, Pyrogen testing, ocular irritation, and stability.

Appearance, pH and drug content were almost same for all formulations. On the basis of gelling capacity and viscosity of liquid formulations three formulations (TF2, TF3 & TF4) were selected and subjected for further evaluation.

All the three formulations showed the constant drug release for a period of 6 Hrs. PF8 showed the highest cumulative drug release due to most accurate concentration of Poloxamer 407, Carbopol and PEG 400.

Release kinetics studies showed that Formulations released the drug in a controlled manner following zero order controlled release. The non-Fickian (anomalous) release kinetics, revealed by (*n*) values between 0.57 and 0.84, might indicate that the release of Levofloxacin hemihydrate followed coupled erosion-diffusion mechanism.

Concerning the viscosity of the formulated *temperature sensitive* gels, the mucoadhesive polymers substantially contributed to the shear viscosity of the thermo-sensitive gels with a increase in the consistency index (*m*) in case of Carbopol containing gel compared to PVA containing gels while the addition of PEG decreased the shear viscosity of Poloxamer formulations which would agree with the results reported by Edsman et al. (1998) [14].

All the three formulations passed the tests for sterility, Pyrogenicity, Ocular irritation and stability, showing that the developed formulations are sterile, stable, non-irritant and therapeutically efficacious.

CONCLUSION

In this study the Temperature sensitive ophthalmic hydrogels of Levofloxacin hemihydrate with enhanced solubility, as well as prolonged retention time, were obtained by the combination of Poloxamer and other polymers. Study showed that the prepared formulations were liquid at room temperature and underwent rapid gelation upon instillation into the eyes. β-cyclodextrin increased the solubility of Levofloxacin to a considerable extent. All formulations showed sustained drug release over 6-h period of time. Hence, this formulation can be an alternate to conventional eye drops to improve the bioavailability through its longer preocular residence time and ability to sustain drug release. Nevertheless, the most prominent advantage of the *Temperature sensitive hydrogels* over the conventional eye drops and silent gels is that it is fluid-like prior to contact with the conjunctival membrane: a feature that is warranted for convenience of administration for patients, accuracy of drug dosing and avoidance of the blurring of vision in case of normal gels. In conclusion of all experimental results it can be stated that present work was a satisfactory preliminary study in developing *Temperature Sensitive Ophthalmic Hydrogels* of Levofloxacin hemihydrate. Further detailed investigations needed towards the optimization of concentration of gelling and mucoadhesive agent to formulate the Stimuli Sensitive Hydrogels system for ophthalmic delivery. The *in-vivo* release studies and *in vitro* – *in vivo* correlation need to be established to guarantee the bioavailability of prepared formulations.

ACKNOWLEDGEMENTS

Two of authors are grateful to Department of Pharmacy, Pranveer Singh Institute of Technology, Kanpur (U.P) for providing necessary facilities required to carry out the project work.

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