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PH SENSITIVE HYDROGELS OF LEVOFLOXACIN HEMIHYDRATE FOR OPHTHALMIC DRUG DELIVERY

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ABSTRACT

To achieve effective ophthalmic therapy, an adequate amount of active ingredient must be delivered and maintained within the eye. The most frequently used dosage forms i.e., eye solutions, eye ointments, eye gels and eye suspensions are compromised in their effectiveness by several limitations leading to poor ocular bioavailability**.** ***Stimuli sensitive hydrogels*** are of very importance in this regard, they undergo reversible volume and/or sol-gel phase transitions in response to physiological *(temperature, pH and presence of ions in organism fluids, enzyme substrate)* or other external (electric current, light) stimuli. They help to increase in precorneal residence time of drug to a sufficient extent that an ocularly delivered drug can exhibit its maximum biological action. The present work describes the formulation and evaluation of pH sensitive ophthalmic Hydrogels of an Antibacterial agent, **"Levofloxacin hemihydrate"**. "Levofloxacin" is a widely used drug for treatment of acute conjunctivitis. However Conventional ophthalmic dosage forms of Levofloxacin generally suffer the drawbacks of poor bioavailability due to its pH dependent solubility. To increase the solubility of Levofloxacin, the pH Sensitive ophthalmic hydrogels of the drug were formulated along with β-Cyclodextrin. Carbopol 940 was used as the gelling agent in combination with Hydroxypropylmethylcellulose (+3000cps) which acted as a viscosity enhancing agent. The developed formulation was therapeutically efficacious, stable, and non-irritant and provided sustained release of the drug over a 6-h period. The developed system is thus a viable alternative to conventional eye drops.

Key Words: Ophthalmic; pH sensitive; hydrogel; Levofloxacin; instillation; viscosity

INTRODUCTION

Eye is most interesting organ due to its drug disposition characteristics. Amongst the

various routes of drug delivery, the field of ocular drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. Compared with drug delivery to other parts of the body, drug delivery to the eye has met with significant challenges posed by various ocular barriers. Many of these barriers are inherent and unique to ocular anatomy and physiology making it a challenging task for drug delivery scientists. These barriers are specific depending upon the route of administration viz. topical, systemic, and injectable. Most of these are anatomical and physiological barriers that normally protect the eye from toxicants. [1]

In the ophthalmic drug delivery systems, the physiological constraints imposed by the protective mechanism of the eye lead to the low absorption of drugs and results in a short duration of therapeutic action. A high frequency of the eye drops instillation is associated with patient's non compliance. After the instillation of eye drop into the eye cavity, the effective tear drainage and blinking action of eye results in a 10 times reduction in the drug concentration within 4- 20 min.[2] Due to the tear drainage, most of the administered dose passes via nasolacrimal duct into the Gastro Intestinal tract leading to the side effects. Rapid elimination of the eye drops often results in a short duration of the therapeutic effect .The normal volume of tear in the eye is 7 µl where as a non blinking eye can accommodate a maximum of 30 µl of the fluid, blinking eye can hold only 10µl both normally & externally added solution are rapidly drained from eye. The usual single drop size of an instilled drug solution is up to 50 µl & thus most of the drug instilled as eye drop is lost.

Ophthalmic therapy can be improved by increasing the corneal residence time of drugs. The preceding summary demonstrates that the formulator faces many constraints and prerequisites when developing a modified-release topical ophthalmic drug. Development of newer, more sensitive diagnostic techniques and novel therapeutic agents continue to provide ocular delivery systems with high therapeutic efficacy. Conventional ophthalmic formulations like solution, suspension, and ointment have many disadvantages which result into poor bioavailability of drug in the ocular cavity. All these conditions nesseciates the development of a specific dosage form for ophthalmic drug delivery, known as Hydrogel systems.

Hydrogels can be defined as Polymers endowed with the ability to swell in water or aqueous solvents and induce a sol-gel transition. They resemble natural living tissue more than any other class of synthetic biomaterials due to their high water content; furthermore, the high water content of the materials contributes to their biocompatibility.[3] In this regard, the

phase-change polymers, which may trigger drug release in response to external stimuli, are the most investigated. Hydrogels providing such 'sensor' properties are referred as *Stimuli sensitive hydrogels* or smart hydrogels. These "intelligent" or "smart" polymers play important role in drug delivery since they may dictate not only where a drug is delivered, but also when and with which interval it is released. The stimuli that induce various responses of the hydrogels systems include physical (temperature) or chemical (pH, ions) stimuli. [4]

Cellulose acetate phthalate (CAP) latex, cross linked acrylic, and derivatives such as Carbomer are used for the preparation of pHsensitive Hydrogels.[5] Cellulose acetate derivatives are the only polymer known to have a buffer capacity that is low enough to gel effectively in the cul-de-sac of the eye. The pH change of about 2.8 units after instillation of the native formulation (pH 4.4) into the tear film leads to an almost instantaneous transformation of the highly fluid latex into viscous gel. The gamma scintigraphy technique was used to monitor the ocular residence time of an ophthalmic preparation based on Cellulose acetate phthalate (CAP) dispersion. The gelled system constitutes a micro-reservoir of high viscosity. [6, 7]

Srividya, et al. studied ophthalmic delivery based on pH-triggered *in situ* gelling and showed *in vitro* release of more than 8-hour period.[8] The developed system was stable, non-irritant, and a viable alternative to 5 conventional eye drops. Chunjie Wu et al. investigated pH-triggered in situ gelling system of puerarin and showed better pseudoplastic behavior of the fluid and *in vitro* release of gelling system was better than puerarin eye drops.[9] Zhidong Liu, et al. investigated the formulation and evaluation of an ophthalmic delivery system of an antibacterial agent, gatifloxacin based on *in situ* gelling system and showed improved rheological behaviour, enhanced ocular bioavailability and better patient compliance compared to conventional ophthalmic solutions.[10] Shastri et al. formulated the *in situ* gelling thermoreversible mucoadhesive gels of an antibacterial agent Moxifloxacin HCl using combination of Poloxamer 407 and Poloxamer 188 with different mucoadhesive polymers such as Gelrite.[11] The formulated gels were transparent, uniform in consistency and had spreadability with a pH range of 6.8 to 7.4. Singh et al. formulated the pH Sensitive hydrogel systems of the anti glaucoma agent, Timolol Maleate. Poly acrylic acid (carbopol 934p) was used as a gelling agent in the combination with the Viscolyzers i.e. hydroxyl propylmethyl cellulose.[12] The developed hydrogels were found to be therapeutically effacious, stable, non irritant and provided a sustained release of drug over an 8 hours time period.

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 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 **.** [13]

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.

The objective of present investigation is to increase the solubility of Antibacterial Ophthalmic drug **"Levofloxacin hemihydrate"** by the use of β-Cyclodextrin and to formulate & evaluate the pH Sensitive ophthalmic hydrogels of this drug in view of increasing precorneal residence time & bioavailability of drug.

MATERIALS AND METHODS

Materials

Levofloxacin hemihydrate was obtained as a gift sample from Galpha Laboratories Ltd., Baddi (Himachal Pradesh). Carbopol 940 and HPMC (+3000 cps) were obtained by SD Fine Chemical Ltd., Mumbai. All chemicals used were of analytical grade.

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 pH Sensitive Hydrogel formulations

Experimental design

A 2 level 3 factors factorial design (2^3) was employed to design ocular pH Sensitive Levofloxacin hydrogels. The design was employed for formulations containing different concentrations of gelling agent (Carbopol 940), Viscofying agent (HPMC) & β-CD, but having common proportions of drug and other excipients. Concentration of Gelling agent (Carbopol) and Concentration of Viscofying agent (HPMC) were selected as Independent variables while gelling capacity and Viscosity of liquid formulations were used as dependent variables.

Table 1: Formulation design for pH Sensitive Hydrogels

Contents	Quantity (% w/v)							
	PF1	**PF2**	**PF3**	**PF4**	**PF5**	**PF6**	**PF7**	**PF8**
Drug	0.512	0.512	0.512	0.512	0.512	0.512	0.512	0.512
Carbopol 940	0.4	0.4	0.4	0.4	0.5	0.5	0.5	0.5
HPMC (+3000 cps)	0.5	0.5	0.7	0.7	0.5	0.5	0.7	0.7
β-CD	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2
Benzalkonium chloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sodium chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
2M NaOH	QS	QS	QS	QS	QS	QS	QS	QS
Phosphate buffer pH 5.5	QS 100 ml	QS 100 ml	QS 100 ml	QS 100 ml	QS 100 ml	QS 100 ml	QS 100 ml	QS 100ml

Method of Preparation [14,15,16]

8 Formulations containing different concentrations of Carbopol 940, HPMC (+3000 cps) and β-CD (Table- 1) were prepared and evaluated for gelling capacity and viscosity in order to identify the compositions suitable for use as pH Sensitive hydrogel systems.

The detailed procedure for the preparation of pH Sensitive ophthalmic hydrogels is outlined as below. The required amount of Carbopol 940 was dispersed in Phosphate buffer pH 5.5 with continuous stirring until completely dissolved. Then the required amount of HPMC (+3000 cps) was allowed to hydrate with buffer solution for about 24 hours, to minimize the formation of lumps. After 24 hours both the polymer solutions were mixed together with gentle stirring. Levofloxacin was dissolved in small volume of buffer solution. β-Cyclodextrin, Benzalkonium chloride and sodium chloride were added to drug solution. This drug solution was added to the polymeric mixture and required volume was made up with 5.5 pH phosphate buffer. The pH of formulation was adjusted using sodium hydroxide (2M). Finally, the resultant solution was subjected to membrane filtration by using cellulose membrane of pore size of 0.22µm. 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 120^0C 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 *pH 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 max (287 nm) using UV-spectrophotometer (UV-1700 Pharma Spec, Shimadzu, Kyoto, Japan) to calculate the percentage of drug content . [17]

Gelling capacity

All pH Sensitive gelling formulations were evaluated for gelling capacity to identify the compositions suitable for use. The 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 37^0 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 [18]

All pH Sensitive gelling formulations in liquid form were evaluated for Viscosity. Viscosity of liquid formulations was measured using a Brookfield Synchrolectric 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 pH Sensitive hydrogel formulations.

***In vitro* Release Studies**

The *in vitro* release of Levofloxacin hemihydrate from all selected pH 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 pH 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 10 rpm.

Sterility Testing

IP method (1996) was followed for the sterility testing of pH sensitive eye drops. Sterility testing was carried out by incubating formulations for not less than 15 days at 30 to 35^0 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 pH 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 pH 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 pH Sensitive formulations were subjected to stability studies conditions at 4°C, room temperature (25^oC) with ambient humidity, 37^0C 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 AND DISCUSSION

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.

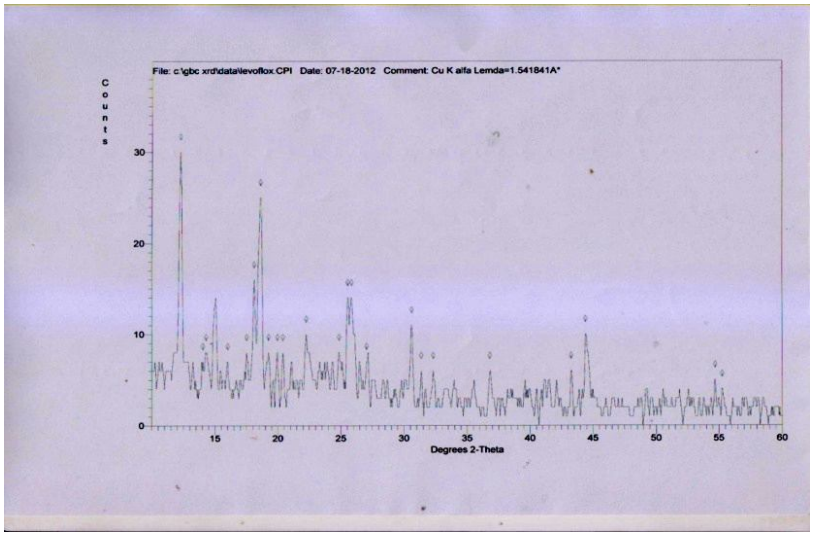

Fig.1: Powder X-ray Diffraction Pattern of Levofloxacin hemihydrate

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.

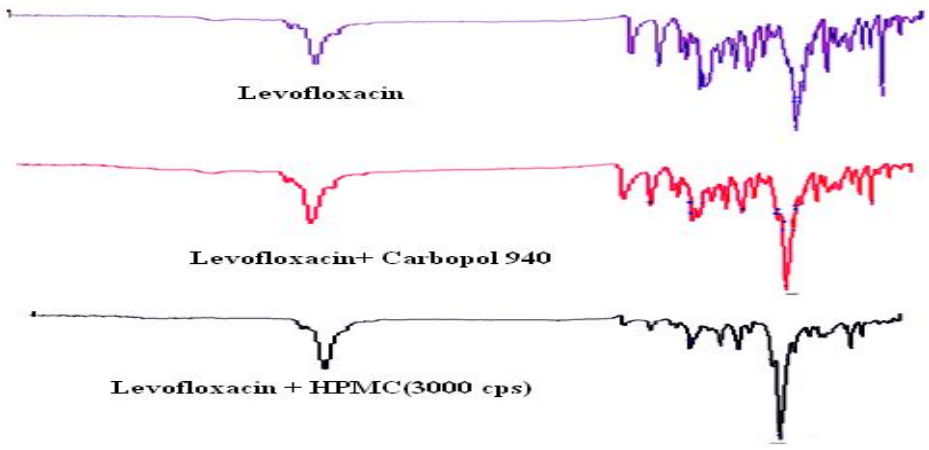

Fig.2: IR Spectra of Pure Drug and Physical mixtures of Drug with Polymers

Preparation of pH Sensitive Ophthalmic Hydrogels

8 Formulations containing Carbopol 940 and HPMC (+3000 cps) in different concentrations were prepared. The use of Carbopol-940 in pH Sensitive Hydrogels is substantiated by the

property of its aqueous solutions to transform into stiff gels when the pH is changed from acidic to basic. HPMC was used to increase the viscosity of formulation & to decrease the concentration of gelling agent. The phosphate buffer pH 5.5 was used as a vehicle in the pH Sensitive gelling system.

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 haziness observed during autoclaving due to precipitation of HPMC at elevated temperature was found to disappear and the clarity was regained after overnight standing. The pH of all the formulations was found to be within the range of 6.0 to 6.4, which is desirable for the ophthalmic formulations. The drug content of all the formulations was within the range of 94.35% to 98.85%, showed the uniform distribution of drug in the ophthalmic formulations Table-2.

Table 2: Appearance, pH and Percent Drug Content for pH Sensitive Hydrogels

pH Sensitive Formulations	Appearance	Clarity	pH	%Drug Content ± S.D
PF1	Light Yellow	Clear	6.1	94.35 ± 0.78
PF2	Light Yellow	Clear	6.0	95.4 ± 0.56
PF3	Light Yellow	Clear	6.3	97.35 ± 0.35
PF4	Light Yellow	Clear	6.1	98.05 ± 0.21
PF5	Light Yellow	Clear	6.2	97.25 ± 0.78
PF6	Light Yellow	Clear	6.0	98.1 ± 0.14
PF7	Light Yellow	Clear	6.2	98.35 ± 0.35
PF8	Light Yellow	Clear	6.1	98.85 ± 0.49

PF1- Carbopol 0.4% w/v HPMC 0.5% w/v β-CD 0.1% w/v, PF2- Carbopol 0.4% w/v HPMC 0.5% w/v β-CD 0.2% w/v, PF3- Carbopol 0.4% w/v HPMC 0.7% w/v β-CD 0.1% w/v, PF4- Carbopol 0.4% w/v HPMC 0.7% w/v β-CD 0.2% w/v, PF5- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.1% w/v, PF6- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.2% w/v, PF7- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.1% w/v, PF8- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.2% w/v.

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-3 shows the gelling capacity of all pH Sensitive formulations. Fig. 3 shows the effect of independent variables on the gelling capacity of all the pH Sensitive formulations. The 3-dimensional response surface plots of Gelling capacity showed that the gelling capacity increases with increasing concentration of gelling agent both at higher and lower concentration of viscofying agent.

All the formulations except PF1 and PF2 showed instantaneous gelation when contacted with simulated tear fluid (STF). However, the nature of the gel formed depended on the concentration of polymers used. The formation of instantaneous gels can be attributed to the buffering capacity of the simulated tear fluid. Formulation PF1 and PF2 showed the formation of gel after a few minutes which dissolved rapidly. Formulation PF3 and PF4 showed immediate gelation and remained for few hours, where as the formulation PF5, PF6, PF7 and PF8 showed immediate gelation and remained for extended period.

Table 3: Results of response variables for 2^3 factorial design in pH Sensitive Hydrogels

pH sensitive Formulation code	Viscosity Of Liquid Formulations at 10rpm Y1 (Pa-s)	Gelling Capacity Y2
PF1	1.645	+
PF2	1.866	+
PF3	2.869	++
PF4	2.993	++
PF5	2.856	+++
PF6	2.947	+++
PF7	3.364	+++
PF8	3.482	+++

PF1- Carbopol 0.4% w/v HPMC 0.5% w/v β-CD 0.1% w/v, PF2- Carbopol 0.4% w/v HPMC 0.5% w/v β-CD 0.2% w/v, PF3- Carbopol 0.4% w/v HPMC 0.7% w/v β-CD 0.1% w/v, PF4- Carbopol 0.4% w/v HPMC 0.7% w/v β-CD 0.2% w/v, PF5- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.1% w/v, PF6- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.2% w/v, PF7- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.1% w/v, PF8- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.2% w/v. +, Gels after a few minutes, dissolves rapidly; ++, Gelation immediate, remains for few hours; +++, Gelation immediate, remains for extended period.

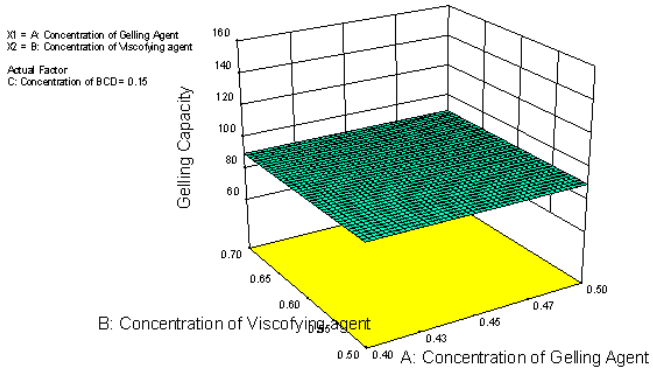

Fig. 3: 3-dimensional response surface plot for Gelling Capacity in pH Sensitive Hydrogels

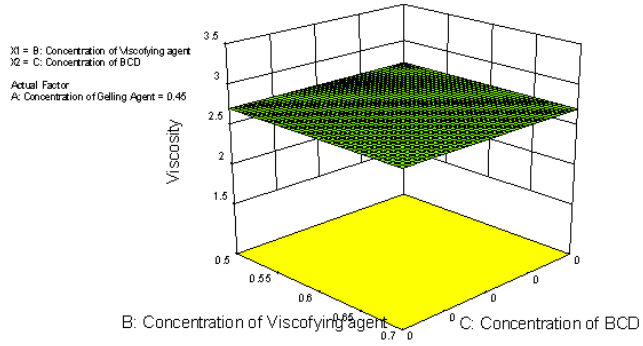

Fig. 4: 3-dimensional response surface plot for viscosity in pH sensitive Hydrogels

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-3 shows the viscosity of all liquid pH Sensitive formulations at 10 rpm. Fig. 4 shows the effects of independent variables on viscosity of pH Sensitive formulations. 3-dimensional Response surface plots of viscosity showed that in all pH Sensitive Hydrogels, the viscosity increased in proportion with viscofying agent both at lower and higher concentration of gelling agent, i.e. gelling agent had a little effect on viscosity.

On the basis of gelling capacity and viscosity PF5, PF6 and PF7 and PF8 showed optimum results within the desired range. Hence, these four pH Sensitive formulations were subjected for further evaluation parameters.

In Vitro Release Studies

The *in-vitro* release profile of the formulations is shown in Table 4.The results indicated that the formulation PF8 showed better sustaining effect amongst all formulations. This may be due to the presence of higher concentration of carbopol 940 along with HPMC (+3000 cps) in the formulation PF8.All formulations showed an initial burst release. The prolonged release in the later stage can be attributed to the slow diffusion of the drug through polymer matrix. The initial burst release of the drug can be explained by the fact that, the *Stimuli Sensitive* system is formulated in water and hence the polymer was completely hydrated. When they come in contact with STF, gelation occurs and a prehydrated matrix is formed in which hydration and water penetration no longer limit drug release, leading to an apparent diffusion-controlled release. 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.

Table 4: *In-vitro* Release profile for selected pH Sensitive Hydrogels

Time (Hrs)	Percent Drug Released (%)			
	PF5	PF6	PF7	PF8
0.25	14.48	16.71	17.44	17.96
0.5	20.09	20.40	23.47	21.25
1	25.38	24.21	29.18	29.82
1.5	31.30	29.93	35.74	36.69
2	36.59	33.52	41.57	41.56
2.5	42.20	40.29	46.33	47.28
3	47.59	46.75	52.68	54.68
3.5	53.42	51.83	58.81	59.76
4	58.92	55.53	63.36	65.37
4.5	66.22	59.34	69.18	71.19
5	71.40	65.48	74.47	76.48
5.5	75.21	68.76	81.77	83.68
6	78.39	79.97	84.31	86.22

PF5- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.1% w/v, PF6- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.2% w/v, PF7- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.1% w/v, PF8- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.2% w/v.

Drug Release Kinetics Studies

Plots of zero order, first order, Higuchi matrix and Hixson Crowell for pH Sensitive Hydrogels are depicted in Fig. 5-8. For all the formulations, the best fit model was Zero order

suggesting diffusion controlled release, may be due to the swelling nature of polymers. The 'n' value obtained from Peppas equation were less than 0.5,which indicated that all the formulations showed drug release by Fickian diffusion mechanism. The results are shown in Table 5.

Table-5: Model fitting for the Release Profile of pH Sensitive Formulations by Using 5 Different Models

pHsensitive Formulations	Zero Order	First Order	Higuchi Matrix	Korsmeyer -Peppas		Hixson Crowell	Best Fit	Transport Mechanism Model
	R	R	R	R	n	R		
PF5	0.998	0.989	0.976	0.992	0.495	0.995	Zero	Ficknian
PF6	0.997	0.974	0.979	0.978	0.452	0.983	Zero	Ficknian
PF7	0.998	0.986	0.991	0.986	0.451	0.996	Zero	Ficknian
PF8	0.998	0.979	0.990	0.991	0.465	0.992	Zero	Ficknian

PF5- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.1% w/v, PF6- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.2% w/v, PF7- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.1% w/v, PF8- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.2% w/v.

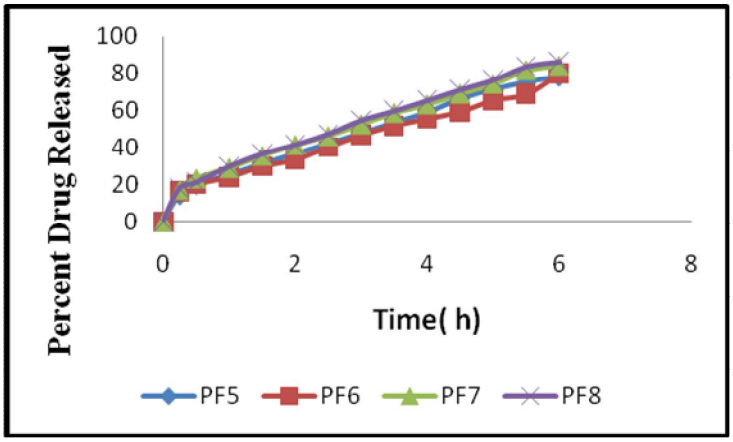

Fig. 5: *In Vitro* Release Profile of Levofloxacin hemihydrate from selected pH Sensitive Hydrogel Formulations. (Zero Order)

PF5- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.1% w/v (—◆—), PF6- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.2% w/v (—■—), PF7- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.1% w/v (—▲—), PF8- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.2% w/v(—✕—).

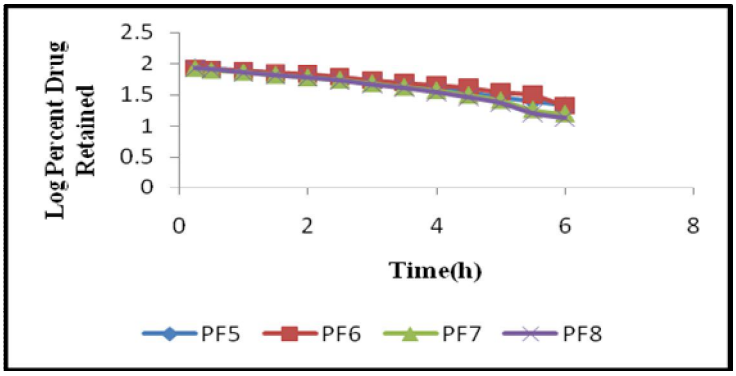

Fig. 6: *In Vitro* Release Profile of Levofloxacin hemihydrate from selected pH Sensitive Hydrogel Formulations. (First Order)

PF5- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.1% w/v (◆), PF6- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.2% w/v (■), PF7- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.1% w/v (▲), PF8- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.2% w/v(✕).

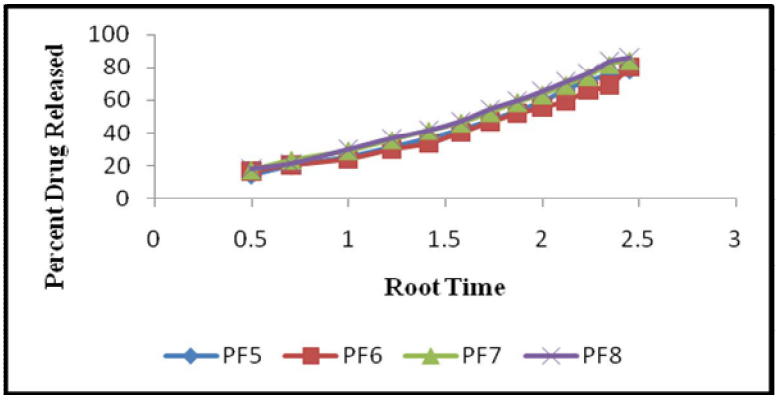

Fig. 7: *In Vitro* Release Profile of Levofloxacin hemihydrate from selected pH Sensitive Hydrogel Formulations. (Higuchi Matrix)

PF5- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.1% w/v (◆), PF6- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.2% w/v (■), PF7- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.1% w/v (▲), PF8- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.2% w/v(✕).

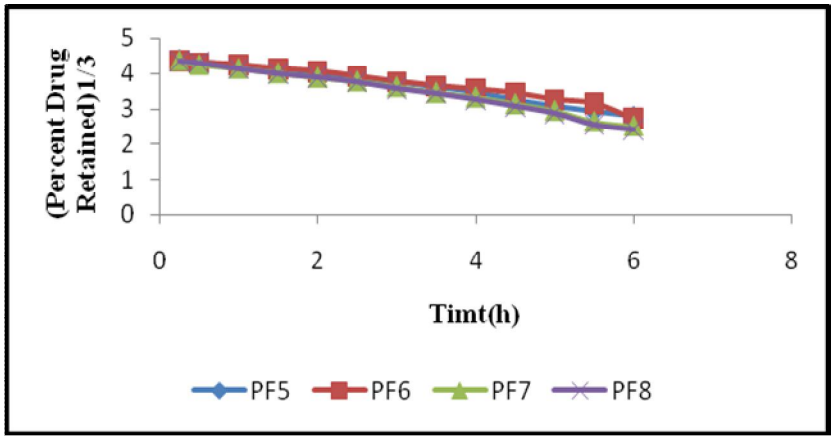

Fig. 8: *In Vitro* Release Profile of Levofloxacin hemihydrate from selected pH Sensitive Hydrogel Formulations. (Hixson Crowell)

PF5- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.1% w/v (), PF6- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.2% w/v (), PF7- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.1% w/v (), PF8- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.2% w/v().

Viscosity Studies of Gelled Formulations

Table 6 shows the viscosity values obtained for selected pH Sensitive (PF5, PF6, PF7 and PF8) 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. The viscosity values of gelled pHsensitive Hydrogel systems of Levofloxacin Hemihydrate are shown in Fig.9. Viscosity study showed that at pH 6.0, the formulations were in a liquid state and exhibited low viscosity. An increase in pH to 7.4 (the pH of the tear fluid) caused the solutions to transform into gels with high viscosity.

Table 6: Rheological Study of pH Sensitive Hydrogel

pH Sensitive Formulations	*Viscosity of Gelled Formulations at 10 rpm (Pa-S)*
PF5	*32.523*
PF6	*34.448*
PF7	*36.587*
PF8	*38.234*

PF5- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.1% w/v, PF6- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.2% w/v, PF7- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.1% w/v, PF8- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.2% w/v.

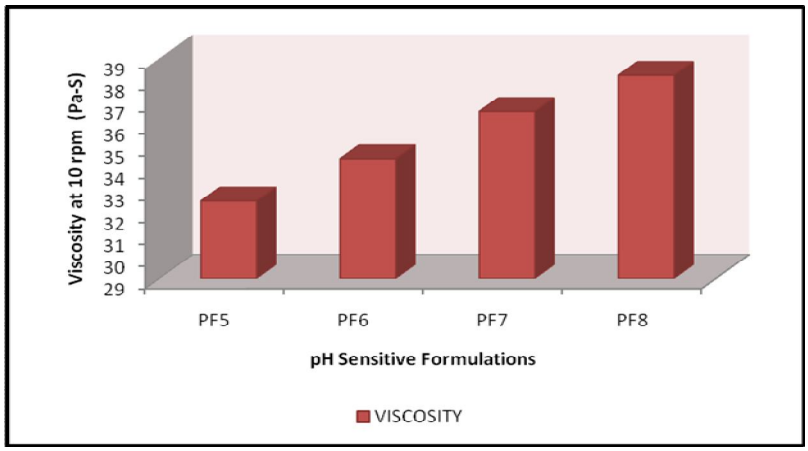

Fig. 9: Viscosity Study of gelled pH Sensitive Formulations (At 10 rpm)

PF5- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.1% w/v, PF6- Carbopol 0.5% w/v HPMC 0.5% w/v β-CD 0.2% w/v, PF7- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.1% w/v, PF8- Carbopol 0.5% w/v HPMC 0.7% w/v β-CD 0.2% w/v.

Sterility Testing

There was no appearance of turbidity and hence no evidence of microbial growth when the formulations were incubated for 15 days at 37^0C temperature and 75% relative humidity in Liquid Nutrient broth media. The preparations being examined therefore passed the test for sterility. (Fig.10).

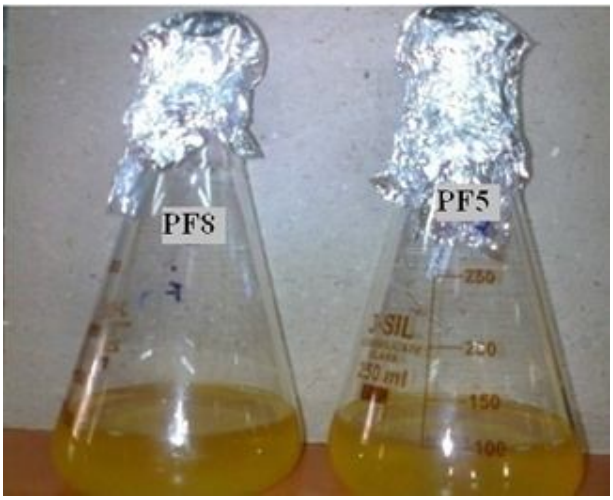

Fig. 10: Sterility test for Levofloxacin pH Sensitive Hydrogels in Liquid Nutrient Broth Media

Pyrogen Testing

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

Ocular Irritation Studies

The results of the ocular irritation studies indicated that all pH sensitive hydrogel formulations were non-irritant. Excellent ocular tolerance was noticed for all the five 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 Stimuli Sensitive formulations of Levofloxacin hemihydrate remained stable at ambient temperature (25^0C) and humidity.

CONCLUSION

The novel ophthalmic pH-sensitive hydrogels of Levofloxacin hemihydrate were successfully formulated by using carbopol 940 and HPMC (+3000 cps). The formulated pH sensitive hydrogel systems were characterized for appearance, clarity, pH, gelling capacity, viscosity and in vitro release in simulated tear fluid. The formulation was liquid at the formulated pH (5.8) and underwent rapid gelation upon raising the pH to 7.4. β-cyclodextrin increased the solubility of Levofloxacin to a considerable extent. The pH sensitive hydrogel system showed sustained drug release over 6-h period of time. So, this formulation is an alternate to conventional eye drops to improve the bioavailability through its longer precorneal residence time and ability to sustain drug release. In conclusion of all experimental results it can be stated that present work was a satisfactory preliminary study in developing *pH Sensitive Ophthalmic Hydrogels* of **Levofloxacin hemihydrate**. Further detailed investigations needed towards the optimization of concentration of gelling and viscofying 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.

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